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(54) Title: PEPTIDES WITH $\beta 1$ INTEGRIN SUBUNIT DEPENDENT CELL ADHESION MODULATING ACTIVITY

(57) Abstract

Peptides capable of modulating $\beta 1$ integrin subunit dependent cell adhesion which includes a C-terminal aromatic amino acid residue and an amino acid residue having a lipophilic alkyl side chain as the penultimate C-terminal residue are provided. These "LipAr" C-terminated peptides are typically capable of modulating the $\beta 1$ integrin subunit dependent adhesion of cells, such as Ramos cells.

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**PEPTIDES WITH $\beta 1$ INTEGRIN SUBUNIT DEPENDENT
CELL ADHESION MODULATING ACTIVITY**

Cross-Referenced to Related Applications

5 The present application claims priority to U.S. provisional application Serial No. 60/072,119 filed on 22 January 1998, entitled "Peptides with Beta Integrin Subunit Dependent Cell Adhesion Modulating Activity"; U.S. provisional application Serial No. 60/096,212 filed on 12 August 1998 entitled "Peptides with $\beta 1$ Integrin Subunit Dependent Cell Adhesion Modulating Activity"; and U.S.
10 provisional application Serial No. 60/096,211 filed on 12 August 1998 entitled "Peptides with $\beta 1$ Integrin Subunit Dependent Cell Adhesion Modulating Activity", the disclosures of which are herein incorporated by reference.

Background of the Invention

 Cellular recognition of the extracellular matrix ("ECM") proteins and of
15 other cells has a complex molecular basis, involving multiple distinct cell surface receptors. Integrins are a family of receptors that are fundamentally important for mediating cell adhesion to ECM proteins. Tumor cells adhere to variety of ECM proteins and molecules on other cells as they invade and metastasize. These interactions of tumor cells have a profound effect on their phenotype. although its
20 exact role is complex and not completely understood, $\alpha 4 \beta 1$ integrin has been implicated in tumor cell arrest and/or extravasation and is involved in tumor cell invasion and metastasis. This integrin is expressed on many hematopoietic malignancies and also on tumors such as melanomas. $\alpha 4 \beta 1$ integrin is unique among integrins in that it binds to both ECM components (e.g. fibronectin) and Ig
25 superfamily adhesion receptors (e.g., VCAM-1) which are expressed on activated endothelial cells and other cell types. $\alpha 4 \beta 1$ integrin also binds to itself and promotes homotypic cell adhesion. Although a role for $\alpha 4 \beta 1$ integrin has been established in modulating various aspects of tumor cell biology, the mechanisms by which the function of the $\alpha 4 \beta 1$ integrin is modulated are complex and not well
30 understood. Understanding the nature of such interactions may help to explain cell-type specific behavior on ECM proteins that are often observed with integrins. There is, accordingly, a continuing need to identify peptides capable of modulating $\alpha 4 \beta 1$ dependent cell adhesion as a means of furthering the understanding of the complex interactions involving this integrin.

Summary of the Invention

The present invention relates to peptides capable of modulating $\beta 1$ integrin subunit dependent cell adhesion. The peptides include a C-terminal amino acid residue having a side chain which includes an aromatic group ("Ar-") and an amino acid residue with a lipophilic alkyl side chain group ("Lip-") as the penultimate C-terminal residue. This C-terminal dipeptide sequence is referred to herein as a "LipAr motif." For example, suitable peptides of the invention may include a C-terminal tyrosine residue and an isoleucine residue as the penultimate C-terminal residue, i.e., a C-terminal "IY motif" (Ile-Tyr). While the present peptides may include a relatively large number of amino acid residues, e.g., up to about 100 amino acid residues or more, as disclosed herein even very small peptides which include the LipAr motif, such as the dipeptide Ile-Tyr and the tripeptide Arg-Ile-Tyr, are capable of modulating $\beta 1$ dependent adhesion. The present peptides typically have no more than about 50 and, preferably, no more than about 25 amino acid residues. The LipAr C-terminated peptides are preferably capable of inhibiting the $\beta 1$ integrin subunit dependent adhesion of cells, such as the $\alpha 4 \beta 1$ integrin dependent adhesion of Ramos cells and the $\alpha 5 \beta 1$ integrin dependent adhesion of erythroleukemic cells (e.g., the erythroleukemic cell line K562).

Brief Description of the Drawings

Figure 1 shows a graph of % adhesion of 8A2 stimulated Ramos cells to IIICS-GST as a function of the concentration of a number of alanine knockout analogs of FN-C/H V+Y. FN C/H V+Y and a scrambled variant lacking a C-terminal IY motif ("sV"; RPQIPWARY (SEQ ID NO:2)) were included as controls.

Figure 2 shows a graph of % adhesion of 8A2 stimulated Ramos cells to IIICS-GST as a function of the concentration of a number of alanine knockout analogs of FN-C/H V+Y. FN C/H V+Y and its scrambled analog sV were included as controls.

Figure 3 shows a graph of % adhesion of 8A2 stimulated Ramos cells to IIICS-GST as a function of the concentration of a number of fibronectin fragments tagged with a C-terminal tyrosine residue. FN C/H V+Y and its scrambled analog sV were included as controls.

Figure 4 shows a graph of % adhesion of 8A2 stimulated Ramos cells to IIICS-GST as a function of the concentration of a number of fibronectin fragments tagged with a C-terminal tyrosine residue. FN C/H V+Y and its scrambled analog sV were included as controls.

5 Figure 5 shows a graph of % adhesion of 8A2 stimulated Ramos cells to IIICS-GST as a function of the concentration of two "IY" C-terminated peptides and their corresponding "des-Y" analogs. FN C/H V+Y and its scrambled analog sV were included as controls.

10 Figure 6 shows a graph of % adhesion of 8A2 stimulated Ramos cells to IIICS-GST as a function of the concentration of another "IY" C-terminated peptide and its corresponding "des-Y" analogs. FN C/H V+Y and its scrambled analog sV were included as controls.

15 Figure 7 shows a graph of % adhesion of 8A2 stimulated Ramos cells to IIICS-GST as a function of the concentration of a number of truncated analogs of FN C/H V+Y. Controls included FN C/H V+Y and its scrambled analog sV.

Figure 8 shows a graph of % adhesion of 8A2 stimulated Ramos cells to IIICS-GST as a function of the concentration of a number of truncated analogs of FN C/H V+Y. FN C/H V+Y and its scrambled analog sV were employed as controls.

20 Figure 9 shows a graph of % adhesion of 8A2 stimulated Ramos cells to IIICS-GST as a function of the concentration of "IY" and its component single amino acid residues. FN C/H V+Y and its scrambled analog sV were employed as controls.

25 Figure 10 shows a graph of % adhesion of 8A2 stimulated Ramos cells to IIICS-GST as a function of the concentration of a several C-terminal penultimate substitution variants of FN C/H V+Y. FN C/H V+Y and its scrambled analog sV were employed as controls.

30 Figure 11 shows a graph of % adhesion of 8A2 stimulated Ramos cells to IIICS-GST as a function of the concentration of a several C-terminal penultimate substitution variants of FN C/H V+Y. FN C/H V+Y and its scrambled analog sV were employed as controls.

Figure 12 shows a graph of % adhesion of 8A2 stimulated Ramos cells to IIICS-GST as a function of the concentration of a several C-terminal substitution variants of FN C/H V+Y. FN C/H V+Y and its scrambled analog sV were employed as controls.

5 Figure 13 shows a graph of % adhesion of 8A2 stimulated Ramos cells to IIICS-GST as a function of the concentration of "IY" positional variants of FN C/H V+Y. FN C/H V+Y, its scrambled analog sV, and untagged FN C/H V (WQPPRARI (SEQ ID NO: 37)) were employed as controls.

10 Figure 14 shows a graph of % adhesion of 8A2 stimulated Ramos cells to IIICS-GST as a function of the concentration of a negatively charged LipAr terminated peptide. FN C/H V+Y and its scrambled analog sV were employed as controls.

15 Figure 15 shows a graph of % adhesion of 8A2 stimulated Ramos cells to IIICS-GST as a function of the concentration of PRARIY (SEQ ID NO: 24) and PRARI (SEQ ID NO: 39). FN C/H V+Y and its scrambled analog sV were employed as controls.

20 Figure 16 shows a graph of % adhesion of the $\alpha 5 \beta 1$ integrin dependent Mn^{+2} stimulated adhesion of erythroleukemic K562 cells to fibronectin ("FN") as a function of the concentration of PRARIY (SEQ ID NO: 24) and PRARI (SEQ ID NO: 39). FN C/H V+Y, its scrambled analog sV, RGD and BSA (bovine serum albumin) were employed as controls.

25 Figure 17 shows a graph of % adhesion of the $\alpha 5 \beta 1$ integrin dependent Mn^{+2} stimulated adhesion of erythroleukemic K562 cells to fibronectin ("FN") as a function of the concentration of RIY. FN C/H V+Y, its scrambled analog sV, RGD, CS1 and BSA (bovine serum albumin) were employed as controls.

Figure 18 shows a graph of % adhesion of the $\alpha 2 \beta 1$, $\alpha 3 \beta 1$ integrin dependent human melanoma M14#5 cell adhesion to laminin ("LM") and type IV collagen ("TIV") and bovine serum albumin ("BSA").

30 Figure 19 shows a graph of % adhesion of 8A2 stimulated Ramos cells to IIICS-GST as a function of the concentration of all D-FN C/H V+Y (SEQ ID NO:1), and a retro inverso form of FN C/H V+Y (SEQ ID NO:40) versus various controls.

Detailed Description of the Invention

The present invention relates to peptides capable of modulating $\beta 1$ integrin subunit dependent cell adhesion. These peptides include a C-terminal LipAr motif and are typically capable of inhibiting $\beta 1$ integrin subunit dependent cell adhesion and, in particular, of inhibiting $\alpha 4\beta 1$ integrin dependent cell adhesion. The present peptides typically are also capable of inhibiting $\alpha 2\beta 1$, $\alpha 3\beta 1$ and/or $\alpha 5\beta 1$ integrin dependent cell adhesion. As used herein, the term "LipAr motif" refers to a dipeptide sequence in which C-terminal "Ar" residue has a side chain which includes an aromatic group. Examples of suitable amino acid residues having an aromatic group include tyrosine ("Tyr"), phenylalanine ("Phe"), histidine ("His"), and tryptophan ("Trp"). The penultimate C-terminal "Lip" residue is an amino acid residue which includes a lipophilic alkyl side chain group. The α -carboxyl group of the C-terminal amino acid residue of the present peptides is typically in the form of a carboxylic acid ($-\text{CO}_2\text{H}$). In a preferred embodiment of the invention, the "Lip" and "Ar" residues are L-amino acid residues.

Examples of amino acid residues which have a lipophilic alkyl side chain group include leucine ("Leu"), isoleucine ("Ile"), and valine ("Val"). Typically, the lipophilic alkyl side chain group has a SCDC (cyclohexane-water side chain distribution coefficient calculated as $-\text{RT} \ln K_D$ and expressed in kcal/mol) of at least about 3.0 and, preferably, at least about 4.0. For the purposes of this application, SCDC is defined according to Radzicka et al., Biochemistry, 27, 1664 (1988). Where the SCDC of a particular alkyl side chain group is not known, the SCDC value may be determined by measurement of the distribution coefficient between wet cyclohexane and water or by a comparison of a compound containing the same alkyl side chain group with other similar compounds using a hydrophobicity scale derived from HPLC retention according to the method of Parker et al., Biochemistry, 25, 5425 (1986). Despite its similarity in some respects to lipophilic alkyl side chain groups such as leucine, isoleucine, and valine, insertion of a methionine residue at the penultimate position (i.e., an "MY" C-terminal motif) resulted in an inactive analog.

Four C-terminal tyrosine tagged peptides having sequences corresponding to different fragments of the fibronectin C-terminal heparin binding domain have been

reported to inhibit the binding of peripheral blood mononuclear cells and spleen cells to fibronectin and endothelial cell monolayers (see, e.g., Wahl et al., J. Clin. Invest., 94, 655-662 (1994)). Two of these peptides, FN-C/H I+Y and FN-C/H V+Y, contain a C-terminal LipAr motif. The amino acid sequence of FN-C/H I+Y is

5 YEKPGSPPREV-VPRPRPGVY (SEQ ID NO:38). The amino acid sequence of FN-C/H V+Y is WQPPRARIY (SEQ ID NO:1). The other two Tyr-tagged fibronectin C-terminal heparin binding domain related peptides do not contain a C-terminal LipAr motif (both peptides end in "TY" (Thr-Tyr)). The amino acid sequences of the these other two fibronectin C-terminal heparin binding domain

10 fragments are KNNQKSEPLIGR-KKTY (FN-C/H II+Y; (SEQ ID NO:39)), and SPPRRARVTY (FN-C/H IV+Y; (SEQ ID NO:40)). Although all four Y-tagged fragments inhibit leukocyte adhesion to fibronectin *in vitro*, only three of the four, FN-C/H I+Y, FN-C/H II+Y and FN-C/H V+Y, are reported to exhibit anti-inflammatory properties in an *in vivo* rat model. One of the four, FN-C/H V+Y, has

15 also been reported to have to inhibit adhesion to VCAM, another extracellular matrix protein. The reported results suggest that the biological activity of the Y-tagged fibronectin C-terminal heparin binding domain fragments is a functional of the specific sequence of each of the peptides.

Several analogs were prepared to examine whether the inhibition of the β 1

20 integrin dependent cell adhesion is effected by the chirality of the inhibitor. The all D-form of FN-C/H V+Y (SEQ ID NO:1) and the all L-form of retro inverso FN-C/H V+Y (SEQ ID NO:40; the all L-form of YIRARPPQW, the reverse primary sequence of FN-C/H V+Y) were prepared and examined in the 8A2 stimulated Ramos cell adhesion assay. Neither of these two compounds inhibited Ramos cell

25 binding, suggesting that the present peptides preferably include the C-terminal LipAr motif in the form of L-enantiomeric amino acid residues.

It has surprisingly been discovered, however, that the alanine knockout analogs of FN-C/H V+Y which preserve the C-terminal LipAr motif (i.e., retain the C-terminal Ile-Tyr dipeptide sequence) are capable of inhibiting β 1 integrin

30 dependent cell adhesion. As used herein, the term "alanine knockout analog" refers to an analog of a peptide in which a single residue has been substituted by an alanine residue. Two of the alanine knockout analogs of FN-C/H V+Y have an alanine

residue substituted for one of the arginine residues in the "PRARI" motif (Pro-Arg-Ala-Arg-Ile (SEQ ID NO:41)) within FN-C/H V+Y which has previously demonstrated to be the implicated in stimulated focal contact formation (see, e.g., Woods et al., Molec. Biol. Cell, 4, 605-613 (1993)). These alanine knockout
5 analogs have the amino acid sequences WQPPRAAIY (SEQ ID NO: 8) and WQPPAARIY (SEQ ID NO: 17). Two of the other alanine knockout analogs, AQPPRARIY (SEQ ID NO: 3), WAPPRARIY (SEQ ID NO: 4), also differ from FN-C/H V+Y by a non-conservative amino acid substitution (Ala for Trp and Ala for Gln respectively).

10 As the examples described herein demonstrate, peptides which differ from FN-C/H V+Y by a non-conservative amino acid substitution but retain the C-terminal LipAr motif can be capable of modulating $\beta 1$ integrin subunit dependent cell adhesion even if the overall physical properties of the peptide differ substantially from FN-C/H V+Y. For example, an FN-C/H V+Y analog in which
15 the two arginine residues have been replaced by aspartic acid residues inhibits the 8A2 stimulated adhesion of Ramos cells at least as strongly as FN-C/H V+Y. The analog, WQPPDADIY (SEQ ID NO: 38), exhibits this activity even though it has an overall net charge of -2 (in contrast to the +2 net charge of FN-C/H V+Y).

Even more surprising than the fact that non-conservative substitution variants
20 of FN-C/H V+Y retain the capability of inhibiting $\beta 1$ integrin subunit dependent cell adhesion, is the fact that other short Lip Ar C-terminated peptides with little or no sequence homology to FN-C/H V+Y also possess this type of biological activity. The results disclosed herein establish that even peptides with less than 50% homology with the corresponding C-terminal portion of FN-C/H V+Y or FN-C/H
25 I+Y exhibit the capability of inhibiting $\beta 1$ integrin subunit dependent adhesion. Examples of such peptides include ARITGYIY (SEQ ID NO:14), RARITGYIY (SEQ ID NO:13), PRQAWRPIY (SEQ ID NO:18), and RPAPQRWIY (SEQ ID NO:20).

As used herein, the term "% homology" refers to the percentage of amino
30 acid residues of a peptide which are either identical to that of an original peptide sequence or differ from the original peptide sequence solely as a result of a conservative amino acid substitution. For example, the peptide PAIFDRSCGS has

40% identity and 80% homology with respect to the peptide sequence
PKVMERTCDS.

For the purposes of this invention, conservative amino acid substitutions are defined to result from exchange of amino acids residues from within one of the following classes of residues: Class I: Ala, Gly, Ser, Thr, and Pro (representing small aliphatic side chains and hydroxyl group side chains); Class II: Cys, Ser, Thr and Tyr (representing side chains including an -OH or -SH group); Class III: Glu, Asp, Asn and Gln (carboxyl group containing side chains); Class IV: His, Arg and Lys (representing basic side chains); Class V: Ile, Val, Leu, Phe and Met (representing hydrophobic side chains); and Class VI: Phe, Trp, Tyr and His (representing aromatic side chains). The classes also include related amino acids such as 3Hyp and 4Hyp in Class I; homocysteine in Class II; 2-aminoadipic acid, 2-aminopimelic acid, γ -carboxyglutamic acid, β -carboxyaspartic acid, and the corresponding amino acid amides in Class III; ornithine, homoarginine, N-methyl lysine, dimethyl lysine, trimethyl lysine, 2,3-diaminopropionic acid, 2,4-diaminobutyric acid, homoarginine, sarcosine and hydroxylysine in Class IV; substituted phenylalanines, norleucine, norvaline, 2-aminooctanoic acid, 2-aminoheptanoic acid, statine and β -valine in Class V; and naphthylalanines, substituted phenylalanines, tetrahydroisoquinoline-3-carboxylic acid, and halogenated tyrosines in Class VI.

In another embodiment of the present invention, the peptides contain no more than 10 amino acid residues and have a sequence which does not correspond substantially to the amino acid sequence of FN-C/H V+Y. As used herein, the sequence of a particular peptide does not correspond substantially to a reference amino acid sequence, if the particular peptide sequence has less than about 80% identity and preferably less than about 50% homology with the reference sequence.

One group of particularly suitable peptides of the invention are those which include a C-terminal "IYY" motif, i.e., the sequence of the three C-terminal most amino acid residues is Ile-Ile-Tyr. One such peptide contains 9 amino acid residues and has the sequence ARITGYIYY (SEQ ID NO:14).

From a variety of standpoints, including cost, ease of production and overall efficiency, smaller versions of the present peptides can offer many distinct

advantages. Thus, one group of particularly advantageous peptides of the invention include the C-terminal IY motif and contain no more than ten and, preferably, no more than six amino acid residues. In addition to the dipeptide Ile-Tyr, suitable examples of this group include PRARIY (SEQ ID NO:24), RARIY (SEQ ID NO:25), ARIY (SEQ ID NO:26) and RIY.

Synthesis of Peptides

The peptides of the invention may be synthesized by the solid phase method using standard methods based on either t-butyloxycarbonyl (BOC) or 9-fluorenylmethoxy-carbonyl (FMOC) protecting groups. This methodology is described by G.B. Fields et al. in Synthetic Peptides: A User's Guide, W.M. Freeman & Company, New York, NY, pp. 77-183 (1992), the disclosure of which is herein incorporated by reference. Peptide structures and purity can be analyzed by HPLC, and amino acid analysis and sequencing.

The present peptides may also be synthesized via recombinant techniques well known to those skilled in the art. For example, U.S. Patent 5,595,887, the disclosure of which is herein incorporated by reference, describes methods of forming a variety of relatively small peptides through expression of a recombinant gene construct coding for a fusion protein which includes a binding protein and one or more copies of the desired target peptide. After expression, the fusion protein is isolated and cleaved using chemical and/or enzymatic methods to produce the desired target peptide.

The peptides described in the examples herein were synthesized by a solid phase method. Tables I and II show the amino acid sequences of the peptides described in the experiments reported herein. The following standard single letter code abbreviations are used to designate the amino acid residues in the peptides: A - alanine, C - cysteine, D - aspartate, E - glutamate, F - phenylalanine, G - glycine, H - histidine, I - isoleucine, K - lysine, L - leucine, M - methionine, N - asparagine, P - proline, Q - glutamine, R - arginine, S - serine, T - threonine, V - valine, W - tryptophan, Y - tyrosine.

Peptide Carrier Conjugates

The peptides of the present invention may be employed in a monovalent state (i.e., free peptide or a single peptide fragment coupled to a carrier molecule). The peptides may also be employed as conjugates having more than one (same or
5 different) peptide fragment bound to a single carrier molecule. The carrier may be a biological carrier molecule (e.g., a glycosaminoglycan, a proteoglycan, albumin or the like) or a synthetic polymer (e.g., a polyalkyleneglycol or a synthetic chromatography support). Typically, ovalbumin, human serum albumin, other proteins, polyethylene glycol, or the like are employed as the carrier. Such
10 modifications may increase the apparent affinity and/or change the stability of a peptide. The number of peptide fragments associated with or bound to each carrier can vary, but from about 4 to 8 peptide fragments per carrier molecule are typically obtained under standard coupling conditions.

For instance, peptide/carrier molecule conjugates may be prepared by
15 treating a mixture of peptides and carrier molecules with a coupling agent, such as a carbodiimide. The coupling agent may activate a carboxyl group on either the peptide or the carrier molecule so that the carboxyl group can react with a nucleophile (e.g., an amino or hydroxyl group) on the other member of the peptide/carrier molecule, resulting in the covalent linkage of the peptide and the
20 carrier molecule. Preferably, the conjugate includes at least one peptide fragment which is not linked to the carrier molecule through an amide bond with the α -carboxyl group of the C-terminal aromatic amino acid residue of the LipAr-terminated fragment.

For example, conjugates of a peptide coupled to ovalbumin may be prepared
25 by dissolving equal amounts of lyophilized peptide and ovalbumin in a small volume of water. In a second tube, 1-ethyl-3-(3-dimethylamino-propyl)-carbodiimide hydrochloride (EDC; ten times the amount of peptide) is dissolved in a small amount of water. The EDC solution was added to the peptide/ovalbumin mixture and allowed to react for a number of hours. The mixture may then be dialyzed (e.g., into
30 phosphate buffered saline) to obtain a purified solution of peptide/ovalbumin conjugate. Peptide/carrier molecule conjugates prepared by this method typically contain about 4 to 5 peptide fragments per ovalbumin molecule.

The invention will be further described by reference to the following detailed examples. The examples are meant to provide illustration and should not be construed as limiting the scope of the present invention.

5

Examples

Assay for Inhibition of $\alpha 4 \beta 1$ Dependent Cell Adhesion

The assay described below was performed to determine whether specific peptides were capable of inhibiting $\beta 1$ integrin subunit modulated cell adhesion and, in particular, of inhibiting $\alpha 4 \beta 1$ dependent Ramos cell adhesion to IIICS-GST, an $\alpha 4 \beta 1$ ligand. IIICS-GST is recombinantly produced fusion protein which contains a fragment from the type III CS region ("IIICS") of plasma fibronectin fused to glutathione-S-transferase ("GST"). The fibronectin fragment corresponds to fibronectin amino acid residues 1961 to 2039 (sequence numbering for fibronectin as designated in U.S. Patent 4,839,464) and includes the

15 DELPQLVTLPHPNLHGPEILDVPST (SEQ ID NO:29) amino acid sequence ("CS1"; fibronectin residues 1961-1985). A synthetically prepared peptide having the CS1 sequence has been shown to interact with $\alpha 4 \beta 1$ integrin on human lymphocytes and promote cell adhesion but does not bind to heparin. In the assay, a 96-well plate was coated with the substrate IIICS-GST. Ramos cells stimulated with

20 the $\beta 1$ activating monoclonal antibody 8A2 ("Ab 8A2") were preincubated with one of the peptides to be evaluated for their ability to adhere to IIICS-GST.

The fusion protein can be constructed by first using PCR primers to amplify the coding sequence for residues 1961-2039 of plasma fibronectin. The PCR product can be introduced into a suitable bacterial expression vector in frame with the gene

25 for GST. The resulting vector can be transformed and expressed in a suitable host cell, such as *E. Coli*, to produce the fusion protein. If desired, the fusion protein can be purified using a glutathione column. In control experiments in which GST alone was coated onto a 96-well plate, no adhesion of 8A2 activated Ramos cells was observed.

30 A 96-well plate was coated in triplicate with 50 μ l/well of IIICS-GST diluted to 3-5 μ g/ml in PBS containing 1mM CaCl_2 , MgCl_2 ("PBS/cations") and incubated overnight at 37°C. The IIICS-GST solution was removed and the wells were

blocked with 150µl/well of PBS/cations containing 0.3% BSA for 1-2 hours at 37°C. During the assay each well contained 100 µl of Ramos cells (10,000 cells/well) with or without peptide. Ramos cells were washed 3 times in adhesion media (DMEM without phenol red containing 20mM HEPES and 3 mg/ml BSA). Cells were
5 counted and resuspended at 200,000 cells/ml. Concentrated Ramos cells were labeled for 20 minutes at 37°C with 50 µg of the fluorescent label BCECF resuspended in 30 µl of dimethylsulfoxide ("DMSO"). The labeled cells were centrifuged and resuspended in adhesion media at a concentration of 200,000 cells/ml. The cells were activated with the activating Ab 8A2 at a concentration of 2
10 µg/ml purified IgG or 1:1000 culture supernatant.

While the cells were being labeled, inhibiting peptide dilutions were prepared. Lyophilized peptides were weighed and resuspended in adhesion media at a stock concentration of twice the maximal inhibitory concentration. If a peptide was difficult to get into solution, it was initially resuspended in 30 µl of DMSO. If
15 a peptide needed to be suspended in DMSO, all of the peptides in that particular experiment (including the controls) were suspended in 30 µl of DMSO. Of the peptides studied in the examples described herein, only the dipeptide "Ile-Tyr" required the use of this technique. The dose-dependent dilutions of peptides were prepared using adhesion media to dilute the stock peptide. Labeled cells were mixed
20 with peptide dilutions for 5 minutes at 37°C at a final concentration of 100,000 cells/ml and appropriate final peptide concentrations.

The blocking solution was removed from the 96-well plate and the cell/peptide mixture is added at 100µl/well (10,000 cells/well) and incubated for 30 minutes at 37°C. An aliquot of standard cells/peptide (1000 µl) was placed at 37°C
25 for quantitating adhesion. Using aspiration, non-adherent cells were removed from the plate. The standard cells were centrifuged and resuspended in 1000 µl of adhesion media. The standard cells were added to empty wells at 100, 80, 60, 40, 20 and 0 µl/well representing 100%, 80%, 60%, 40%, 20% and 0% adhesion, respectively. The plate fluorescence was read at excitation 485 and emission 530.
30 Cell adhesion was represented as percent input cells remaining adherent and was determined by a standard curve of the fluorescence obtained with the standard cells.

The experimental fluorescence readings were extrapolated from the standard curve to obtain percent adhesion.

Example 1 - Alanine Knockout Analogs of FN-C/H V+Y

5 To determine which amino acid residues were required for the $\alpha 4 \beta 1$ dependent cell adhesion inhibiting activity of FN C/H V+Y, a series of analogs having a single individual residue substituted by alanine were examined. The results are shown in Figures 1 and 2. The only alanine substitution which resulted in loss of the ability to inhibit adhesion was substitution of alanine for the isoleucine residue at
10 the penultimate C-terminal position. All of the other alanine knockout peptides showed cell adhesion inhibition comparable to that of FN C/H V+Y. As a control, a scrambled version of the FN C/H V+Y sequence having a C-terminal tyrosine was also examined (RPQIPWARY (SEQ ID NO:2)). The scrambled sequence, which lacked the C-terminal LipAr motif, did not inhibit cell adhesion.

15 Example 2 - C-Terminal Tyrosine Tagged Fibronectin Fragments

A number of other C-terminal tyrosine tagged fibronectin fragments were also examined. These peptides corresponded to tyrosine tagged 8 residue fibronectin fragments which were incrementally displaced by one amino acid residue towards the C-terminus of fibronectin (SEQ ID NOs 10-16 in Table I). The results are
20 shown in Figures 3 and 4. Unexpectedly, only those peptides which included the C-terminal LipAr motif were active in inhibiting $\alpha 4 \beta 1$ integrin dependent cell adhesion. The most active peptide as far as cell inhibiting activity ended with a C-terminal ILY sequence (-Ile-Ile-Tyr-). The full sequence of this peptide was ARITGYIY (SEQ ID NO:14).
25 The sequences of the other two Y-tagged fibronectin fragments which exhibited $\alpha 4 \beta 1$ integrin dependent cell adhesion inhibition was RARITGYIY (SEQ ID NO:13). The Y-tagged fibronectin fragments with a C-terminal Thr-Tyr ("TY"), Gly-Tyr ("GY"), Tyr-Tyr ("YY") or Lys-Tyr ("KY") motif did not inhibit $\alpha 4 \beta 1$ integrin dependent adhesion of the Ramos cells.

Example 3 - Scrambled IY Tagged Sequences

To examine the effect of the N-terminal seven amino acid sequence on inhibition of $\alpha 4\beta 1$ dependent cell adhesion, three Ile-Tyr C-terminated scrambled versions of FN C/H V+Y were examined. The activity of the eight amino acid des-tyrosine analogs of the two scrambled peptides were also examined as controls. The results shown in Figures 5 and 6 clearly demonstrate that only the "LipAr" C-terminated peptides ARITGYIY (SEQ ID NO:14), PRQAWRPIY (SEQ ID NO:18) and RPAPQRWIY (SEQ ID NO:20) inhibited cell adhesion. In each instance, the identical primary amino acid sequence lacking the C-terminal tyrosine residue did not inhibit Ramos cell adhesion. Although not conclusive, this result strongly suggests that there is little or no requirement for the N-terminal portion of the sequence in order for a peptide with a C-terminal LipAr motif to inhibit $\beta 1$ integrin subunit dependent cell adhesion.

Example 4 - Inhibition by Short IY Terminated Peptides

To establish the minimum size of IY-peptide required for inhibition of $\alpha 4\beta 1$ dependent cell adhesion, a study was carried out on a series of truncated FN C/H V+Y analogs in which the N-terminal residue was systematically deleted. The results are shown in Figures 7 and 8. The data establish that the "IY" dipeptide itself is capable of inhibiting $\alpha 4\beta 1$ integrin dependent cell adhesion. The activity of the dipeptide was less than that observed with a number of longer IY terminated peptides. The cell adhesion inhibiting activity of a 6 residue peptide, PRARIY (SEQ ID NO:24), and a 5 residue peptide, RARIY (SEQ ID NO:25), was comparable on an equimolar basis to that of the 9 residue peptide, Y-tagged FN C/H V. These two shortened peptides both contain two arginine residues ("R") and having a net charge of +2 at neutral pH. Other short IY-terminated peptides with the sequences QPPRARIY (SEQ ID NO:22), PPRARIY (SEQ ID NO:23), ARIY (SEQ ID NO:26) and RIY also exhibited $\alpha 4\beta 1$ integrin dependent cell adhesion inhibition activity. The cell adhesion inhibition activity of ARIY (SEQ ID NO:26) and RIY was comparable on an equimolar basis to that of Y-tagged FN C/H V.

Example 5 - Inhibition of Ile-Tyr versus Ile and/or Tyr

As a control experiment, the activity of the single amino acids, isoleucine and tyrosine, alone and as part of a mixture, was also examined in the cell adhesion inhibition activity. The results shown in Figure 9 establish that even a mixture of the individual amino acids isoleucine and tyrosine is insufficient to inhibit cell adhesion at anything close to the concentration where the dipeptide "Ile-Tyr" is active.

Example 6 - Inhibition by "Xaa-Tyr" Terminated Peptides

To examine the structural requirements of the "LipAr" motif, the inhibition of $\alpha 4 \beta 1$ dependent Ramos cell adhesion was examined for a number of FN C/H V+Y analogs with substitutions at the penultimate C-terminal amino acid residue. The results are shown in Figures 10 and 11. The two analogs with a lipophilic aliphatic side chain residue (Leu or Val) substituted at the penultimate C-terminal position, WQPPRARLY (SEQ ID NO:28) and WQPPRARVY (SEQ ID NO:29), had cell adhesion inhibiting activity comparable to that of FN C/H V+Y. The corresponding analogs with a basic residue (Lys), a hydroxy side chain residue (Thr), a methionine residue (Met) or an alanine residue (Ala) in penultimate C-terminal position were substantially inactive in the assay.

Example 7 - Inhibition by C-Terminal Variants

To examine the structural requirements of the "LipAr" motif, the inhibition of $\alpha 4 \beta 1$ dependent Ramos cell adhesion was examined for a number of FN C/H V+Y analogs with substitutions at the C-terminal amino acid residue. The results are shown in Figure 12. The two analogs with a C-terminal amino acid residue having a side chain which includes an aromatic group (Phe or Trp) at the C-terminal position, WQPPRARIF (SEQ ID NO:32) and WQPPRARIW (SEQ ID NO:33), had cell adhesion inhibiting activity comparable to that of FN C/H V+Y.

Example 8 - Inhibition by C-Terminal Variants

The inhibition of $\alpha 4 \beta 1$ dependent Ramos cell adhesion for a number of FN C/H V+Y analogs with differently positioned IY motifs was examined. The results

are shown in Figure 13. Peptides with the "IY" motif at the N-terminus, IYWQPPRAR (SEQ ID NO:34), or in the middle of the peptide, WQPIYPRAR (SEQ ID NO:35) were inactive in the assay. Switching the order of the Ile and Tyr residues at the C-terminus of an FN C/H V+Y analog, WQPPRARYI (SEQ ID NO:35), also resulted in a peptide which was inactive in the $\alpha 4 \beta 1$ dependent Ramos cell adhesion inhibition assay. Finally, control peptide having the tyrosine tag removed from the C-terminus of FN C/H V+Y, WQPPRARI (SEQ ID NO:35), was also inactive in the assay.

10 Example 9 - Inhibition of Adhesion by a Negatively Charged "LipAr" Peptide

All the the LipAr terminated peptides described in the above examples which were active in the Ramos cell adhesion inhibition assay have a net positive charge. In order to determine whether a net positive charge is required for this activity, an FN-C/H V+Y analog in which the 2 arginines (positively charged) were replaced by aspartic acid residues (negatively charged) was evaluated. Importantly, the C-terminal "LipAr" motif ("IY") was retained in this peptide, WQPPDADIY (SEQ ID NO: 38). Figure 14 clearly demonstrates that substitution of the arginines with aspartic acid residues does not alter the ability of the peptide to inhibit $\beta 1$ integrin subunit dependent adhesion, thereby further demonstrating the importance of the "LipAr" motif to this activity.

Example 10 - Inhibition of adhesion by PRARIY versus PRARI

In an experiment which further demonstrated the correlation of a C-terminal LipAr motif with $\beta 1$ integrin subunit dependent adhesion, the peptide PRARIY (SEQ ID NO: 24) and the corresponding sequence lacking the terminal aromatic residue ("Tyr") were evaluated for their ability to inhibit adhesion in the Ramos cell assay. Consistent with the previous results demonstrating the requirement for a C-terminal "LipAr" motif, PRARIY but not PRARI was able to inhibit $\alpha 4 \beta 1$ mediated Ramos cell adhesion to IIICS-GST (see Figure 15).

Example 11 - Inhibition of $\alpha 5\beta 1$ Integrin Dependent Adhesion

To determine whether inhibition of adhesion by C-terminal isoleucine-tyrosine is restricted to $\alpha 4\beta 1$ integrin adhesion, peptide RIY and peptides ending in isoleucine-tyrosine (PRARIY) and isoleucine (PRARI) were evaluated for the ability to inhibit $\alpha 5\beta 1$ integrin-mediated cell adhesion. The cell adhesion assay was carried out as described above. K562 cells stimulated with 1mM $MnCl_2$ were preincubated with the indicated concentration of peptide and allowed to adhere to FN. The results are shown in Figures 16 and 17, where (V), (SV) represent peptides FN-C/H V-Y and scrambled FN-C/H V-Y, respectively. Each data point represents the mean of triplicate determinations and the error bars represent the standard deviation of the mean. The solid black line represents adhesion to the negative control substrate, BSA.

Adhesion of the erythroleukemic cell line K562, which expresses $\alpha 5\beta 1$ but not $\alpha 4\beta 1$ integrin, to FN is completely inhibited following preincubation with soluble RGD or FN-C/H V-Y at the maximal concentration tested, 0.84 mM (Figures 16, 17). The half-maximal inhibitory concentration for soluble peptides RGD and FN-C/H V-Y was 0.1 mM and 0.2 mM, respectively. Furthermore, addition to peptides RIY or PRARIY, but not PRARI, completely inhibited $\alpha 5\beta 1$ dependent K562 adhesion to FN with at the maximal concentration tested, 0.84 mM. The half-maximal inhibitory concentration of both RIY and PRARIY was approximately 0.2 mM, similar to that observed for RGD and FN-C/H V-Y. These results demonstrate that, like peptide FN-C/H V-Y, the smallest, maximally active peptide RIY and peptides ending in isoleucine-tyrosine, but not isoleucine (PRARI), inhibit $\alpha 5\beta 1$ (in addition to $\alpha 4\beta 1$) integrin-mediated adhesion (see Figures 16 and 17).

Example 12 - Inhibition of $\alpha 2\beta 1$, $\alpha 3\beta 1$ Integrin Dependent Adhesion

An experiment was conducted to examine the ability of FN-C/H V+Y (SEQ ID NO:1) to inhibit $\alpha 2\beta 1$, $\alpha 3\beta 1$ integrin dependent cell adhesion using an assay based on human melanoma cells (M14#5).

Laminin, type IV collagen and BSA were coated overnight in a 96 well microtiter plate at 10 μ g/ml and blocked with 0.3% BSA. M14#5 cells were

preincubated with 0.5 mg/ml of peptide (equivalent to 0.42 mM FN-C/H V and scrambled FN-C/H V and 0.17 mM CSI) and allowed to adhere to substrates for 30 minutes.

- 5 Soluble peptide FN-C/H V inhibited human melanoma M14#5 cell adhesion to laminin and type IV collagen-coated substrates, whereas scrambled FN-C/H V has no effect (see Figure 18). This adhesion is dependent on $\alpha 2\beta 1$ and $\alpha 3\beta 1$ integrin as determined using specific anti-integrin blocking mAbs (data not shown).

Example 13 - Influence of Chirality on Inhibition of $\alpha 4\beta 1$ Integrin Dependent

10 Adhesion

The potential chiral dependence of $\beta 1$ integrin dependent cell adhesion by the present peptides was examined by preparing the all D-form of FN-C/H V+Y (SEQ ID NO:1) and the all L-form of retro inverso FN-C/H V+Y (SEQ ID NO:40; the all L-form of YIRARPPQW, the reverse primary sequence of FN-C/H V+Y).

- 15 These two compounds were examined in the 8A2 stimulated Ramos cell adhesion assay.

The results (shown in Figure 19) show that there is a chiral dependence on the adhesion inhibitory activity of FN-C/H V-Y. This suggests that C-terminal isoleucine-tyrosine should be in the L-enantiomeric form since D-amino acid FN-C/H V-Y and a retro-inverso FN-C/H V-Y (consisting of the L-amino acids in reverse primary sequence) are both unable to inhibit adhesion.

- 20 Ramos cells and the $\beta 1$ integrin stimulatory mAb 8A2 were preincubated with the indicated concentration of synthetic peptide prior to addition to rCS1 coated wells. (V), (sV) represent peptides FN-C/H V-Y and scrambled FN-C/H V-Y, respectively. Each data point represents the mean of triplicate determinations and the error bars represent standard deviation of the mean. Background Ramos adhesion to GST is represented in the solid black line.

Example 14 - Inhibition of $\alpha 1\beta 2$ Integrin Dependent Adhesion

- 30 To determine whether inhibition of adhesion by soluble FN-C/H V is specific for $\beta 1$ integrins, the ability of this peptide to inhibit $\beta 2$ integrin-dependent adhesion was also evaluated. For these studies the adhesion of the B-cell line M16B (which

both express functional $\alpha 4\beta 1$ and $\alpha 1\beta 2$ integrin) to purified rCS1 or recombinant ICAM in the presence of soluble FN-C/H V. As expected, $\alpha 4\beta 1$ integrin-dependent Mn^{+2} stimulated M16B adhesion to rCS1 was completely inhibited by soluble FN-C/H V and CS1. However, $\alpha 1\beta 2$ (LFA-1) integrin dependent adhesion to rICAM was not inhibited by soluble FN-C/H V, although this adhesion can be inhibited by an anti- $\beta 2$ integrin blocking mAb.

The invention has been described with reference to various specific and preferred embodiments and techniques. However, it should be understood that many variations and modifications may be made while remaining within the spirit and scope of the invention.

Table I - Peptide Sequences

	<u>SEQ ID NO:</u>	<u>Amino Acid Sequence</u>	<u>Net Charge</u>
15	2	RPQIPWARY	+2
	3	AQPPRARIY	+2
	4	WAPPRARIY	+2
	5	WQAPRARIY	+2
	6	WQPARARIY	+2
20	7	WQPPAARIY	+1
	8	WQPPRAAIY	+1
	9	WQPPRARAY	+2
	10	QPPRARITY	+2
25	11	PPRARITGY	+2
	12	PRARITGYIY	+2
	13	RARITGYIY	+2
	14	ARITGYIY	+1
	15	RITGYIY	0
30	16	ITGYIY	-1
	17	PRQAWRPI	+2
	18	PRQAWRPIY	+2
	19	RPAPQRWI	+2
	20	RPAPQRWIY	+2
35	21	ARITGYIY	+1
	22	QPPRARIY	+2
	23	PPRARIY	+2
	24	PRARIY	+2
40	25	RARIY	+2
	26	ARIY	+1

Table I (cont.) - Peptide Sequences

	<u>SEQ ID NO:</u>	<u>Amino Acid Sequence</u>	<u>Net Charge</u>
5	27	WQPPRARKY	+1
	28	WQPPRARLY	+2
	29	WQPPRARVY	+2
	30	WQPPRARTY	+2
10	31	WQPPRARMY	+2
	32	WQPPRARIF	+2
	33	WQPPRARIW	+2
	34	IYWQPPRAR	+2
	35	WQPIYPRAR	+2
	36	WQPPRARYI	+2
15	37	WQPPRARI	+2
	38	WQPPDADIY	-2
	39	PRARI	+2
	40	YIRARPPQW	+2

WHAT IS CLAIMED IS:

1. A peptide having no more than six amino acid residues which comprises a C-terminal LipAr motif.
2. The peptide of claim 1 comprising a penultimate C-terminal Lip residue selected from the group consisting of Ile, Val and Leu.
3. The peptide of claim 1 comprising a C-terminal Ar residue selected from the group consisting of Tyr, Phe, His and Trp.
4. The peptide of claim 1 comprising a C-terminal motif selected from the group consisting of Ile-Tyr, Ile-Phe, Ile-Trp, Val-Tyr and Leu-Tyr.
5. The peptide of claim 1 comprising a C-terminal Ile-Ile-Tyr motif.
6. The peptide of claim 1 having the sequence Pro-Arg-Ala-Arg-Ile-Tyr (SEQ ID NO:24), Arg-Ala-Arg-Ile-Tyr (SEQ ID NO:25), Ala-Arg-Ile-Tyr (SEQ ID NO:26), Arg-Ile-Tyr or Ile-Tyr.
7. The peptide of claim 1 wherein said peptide is capable of modulating $\beta 1$ integrin subunit dependent adhesion.
8. The peptide of claim 7 wherein said peptide is capable of inhibiting $\beta 1$ integrin subunit dependent adhesion.
9. The peptide of claim 7 wherein said peptide is capable of modulating $\alpha 4 \beta 1$ integrin dependent adhesion.
10. The peptide of claim 9 wherein said peptide is capable of inhibiting $\alpha 4 \beta 1$ integrin dependent cell adhesion.

11. The peptide of claim 10 wherein said peptide is capable of inhibiting $\alpha 4 \beta 1$ integrin dependent adhesion of Ramos cells to $\alpha 4 \beta 1$ integrin binding fibronectin fragments.
12. A peptide having no more than about 10 amino acid residues which comprises a C-terminal LipAr motif and has no more than about 80% identity with WQPPRARIY (SEQ ID NO:1), wherein said peptide does not contain a D-amino acid residue.
13. The peptide of claim 12 comprising a C-terminal sequence selected from the group consisting of ARITGYIY (SEQ ID NO:14), RARITGYIY (SEQ ID NO:13), PRQAWRPIY (SEQ ID NO:18), RPAPQRWIY (SEQ ID NO:20), and WQPPDADIY (SEQ ID NO: 38)).
14. The peptide of claim 12 having no more than about 50% homology with WQPPRARIY (SEQ ID NO:1).
15. A peptide having no more than about 50 amino acid residues which comprises a C-terminal sequence selected from the group consisting of AQPPRARIY (SEQ ID NO:3), WAPPRARIY (SEQ ID NO:4), WQAPRARIY (SEQ ID NO:5), WQPARARIY (SEQ ID NO:6), WQPPAARIY (SEQ ID NO:7), WQPPRAAIY (SEQ ID NO:8), ARITGYIY (SEQ ID NO:14), RARITGYIY (SEQ ID NO:13), PRQAWRPIY (SEQ ID NO:18), RPAPQRWIY (SEQ ID NO:20), WQPPRARLY (SEQ ID NO:28), WQPPRARVY (SEQ ID NO:29), WQPPRARIF (SEQ ID NO:32), WQPPRARIW (SEQ ID NO:33), and WQPPDADIY (SEQ ID NO: 38).
16. The peptide of claim 15 having the sequence AQPPRARIY (SEQ ID NO:3), WAPPRARIY (SEQ ID NO:4), WQPPAARIY (SEQ ID NO:7) or WQPPRAAIY (SEQ ID NO:8).

17. The peptide of claim 15 having the sequence WQAPRARIY (SEQ ID NO:5) or WQPARARIY (SEQ ID NO:6).
18. The peptide of claim 15 having the sequence ARITGYIY (SEQ ID NO:14), or RARITGYIY (SEQ ID NO:13).
19. The peptide of claim 15 having the sequence PRQAWRPIY (SEQ ID NO:18), or RPAPQRWIY (SEQ ID NO:20).
20. The peptide of claim 15 having the sequence WQPPRARLY (SEQ ID NO:28), WQPPRARVY (SEQ ID NO:29), WQPPRARIF (SEQ ID NO:32), or WQPPRARIW (SEQ ID NO:33).
21. The peptide of claim 15 having the sequence WQPPDADIY (SEQ ID NO:38).
22. The peptide of claim 15 having no more than about 15 amino acid residues.
23. A method for modulating the adhesion of cells to a substrate comprising:
 - combining a peptide with a suspension of said cells to form a modified cell suspension, wherein the peptide has no more than about 6 amino acid residues and comprises a C-terminal LipAr motif; and
 - contacting the modified cell suspension with the substrate.

FIG. 1
Inhibition of Ramos adhesion to rCS1
using alanine walkthroughs of FN-C/H V

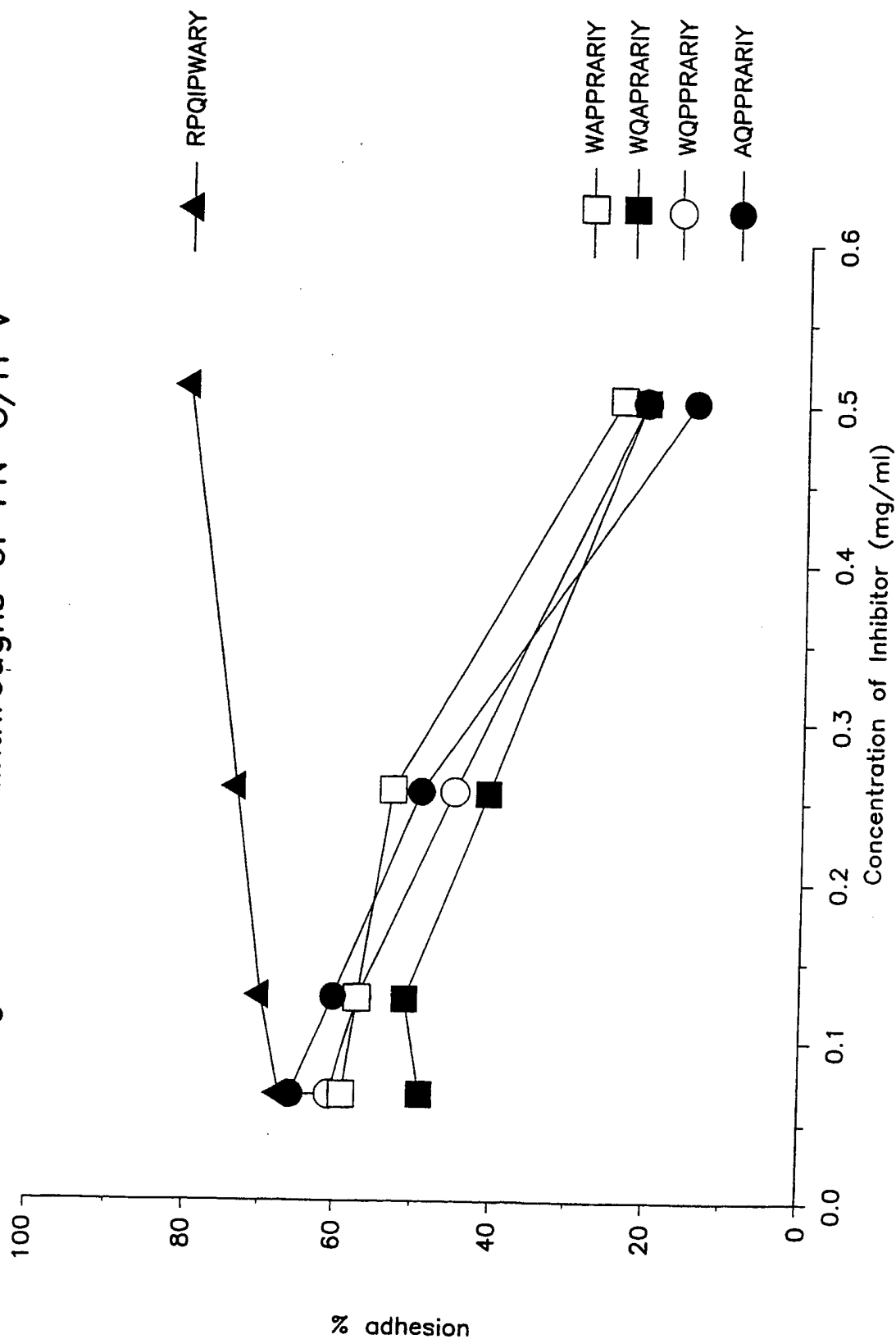


FIG. 2
Inhibition of Ramos adhesion to rCS1
using alanine walkthroughs of FN-C/H V

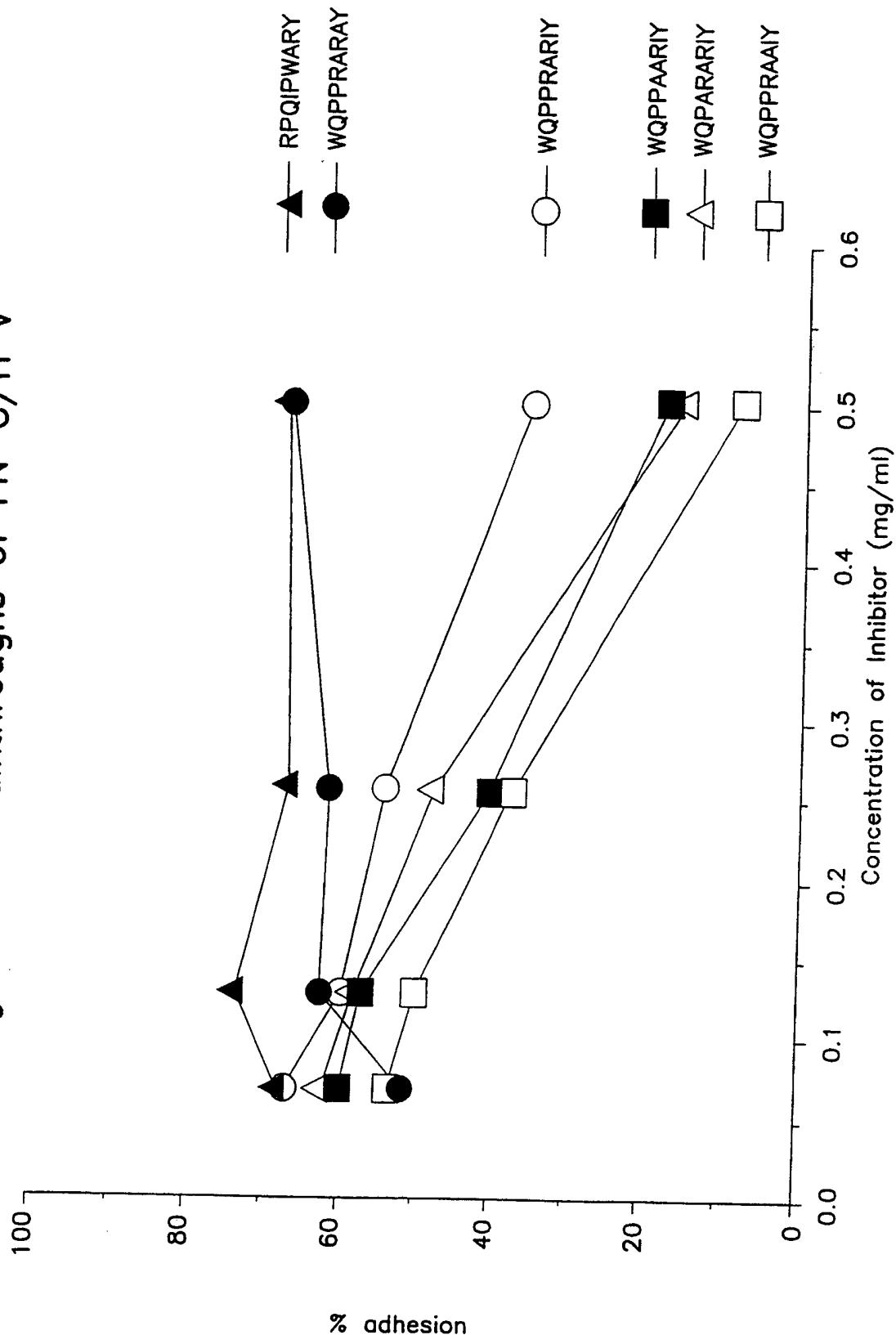


FIG. 3
Inhibition of Ramos adhesion to rCS1 using
8 aa extensions of peptide FN-C/H V

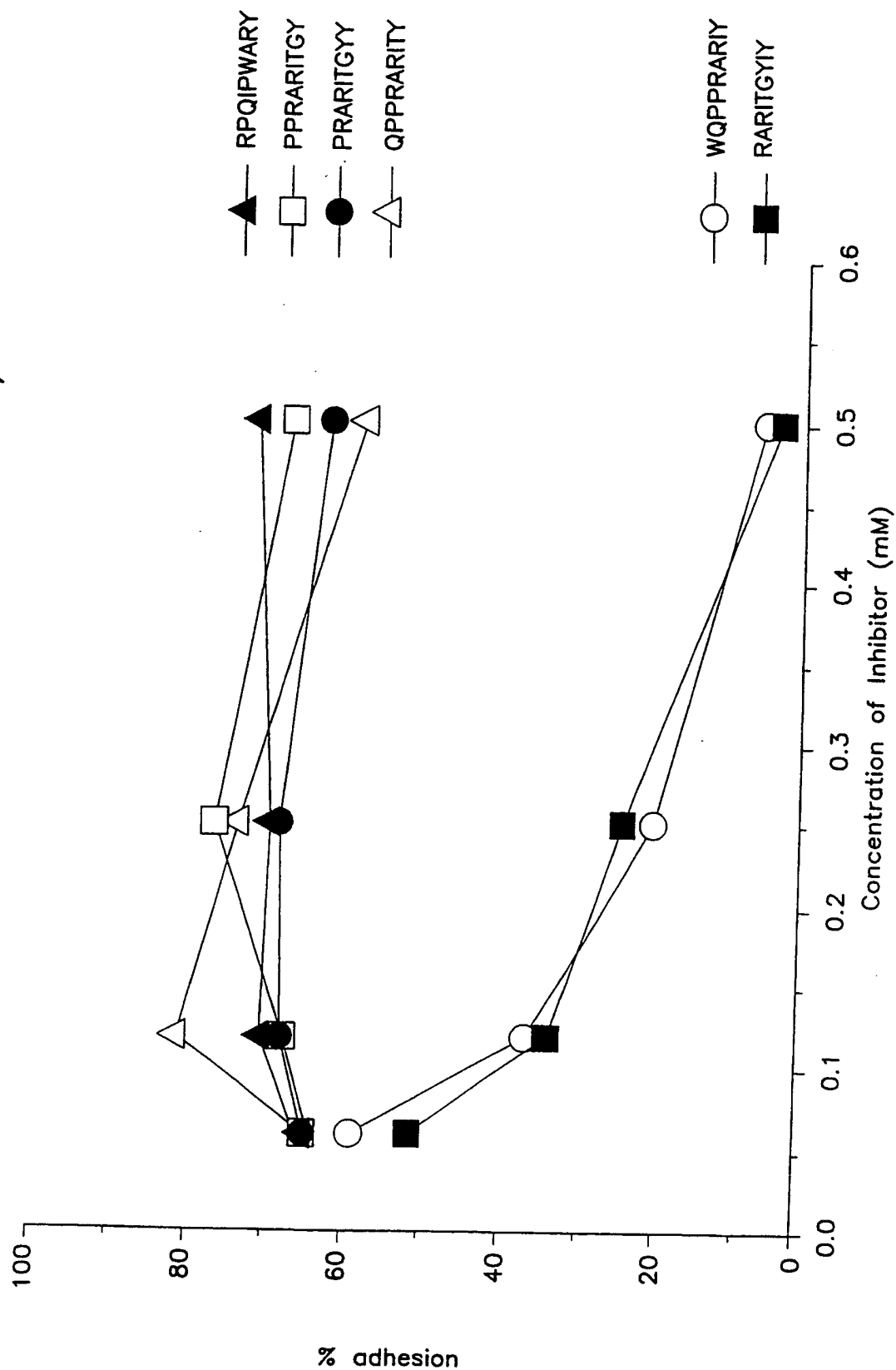
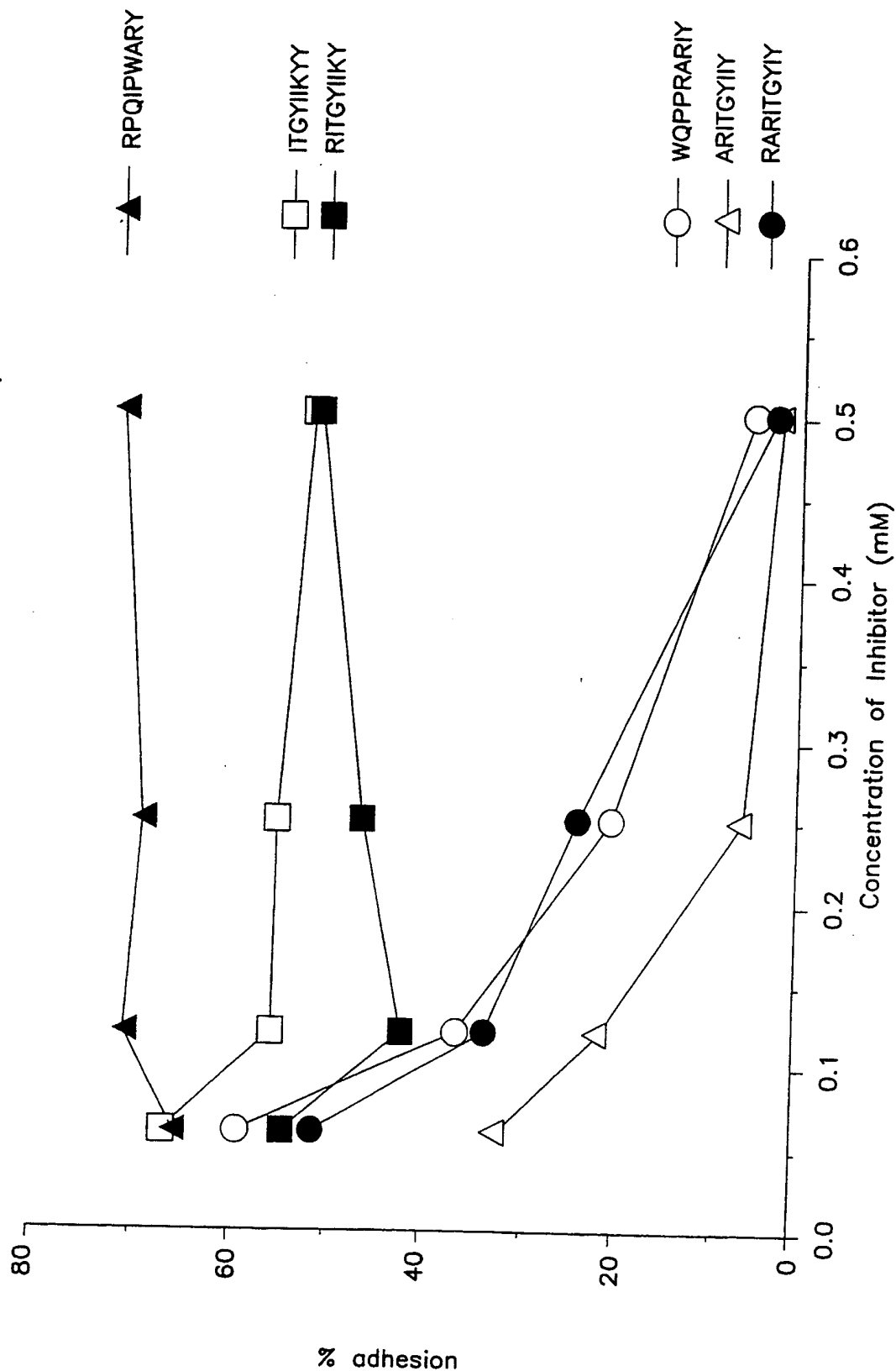


FIG. 4
Inhibition of Ramos adhesion to rCS1 using
8 aa extensions of peptide FN-C/H V



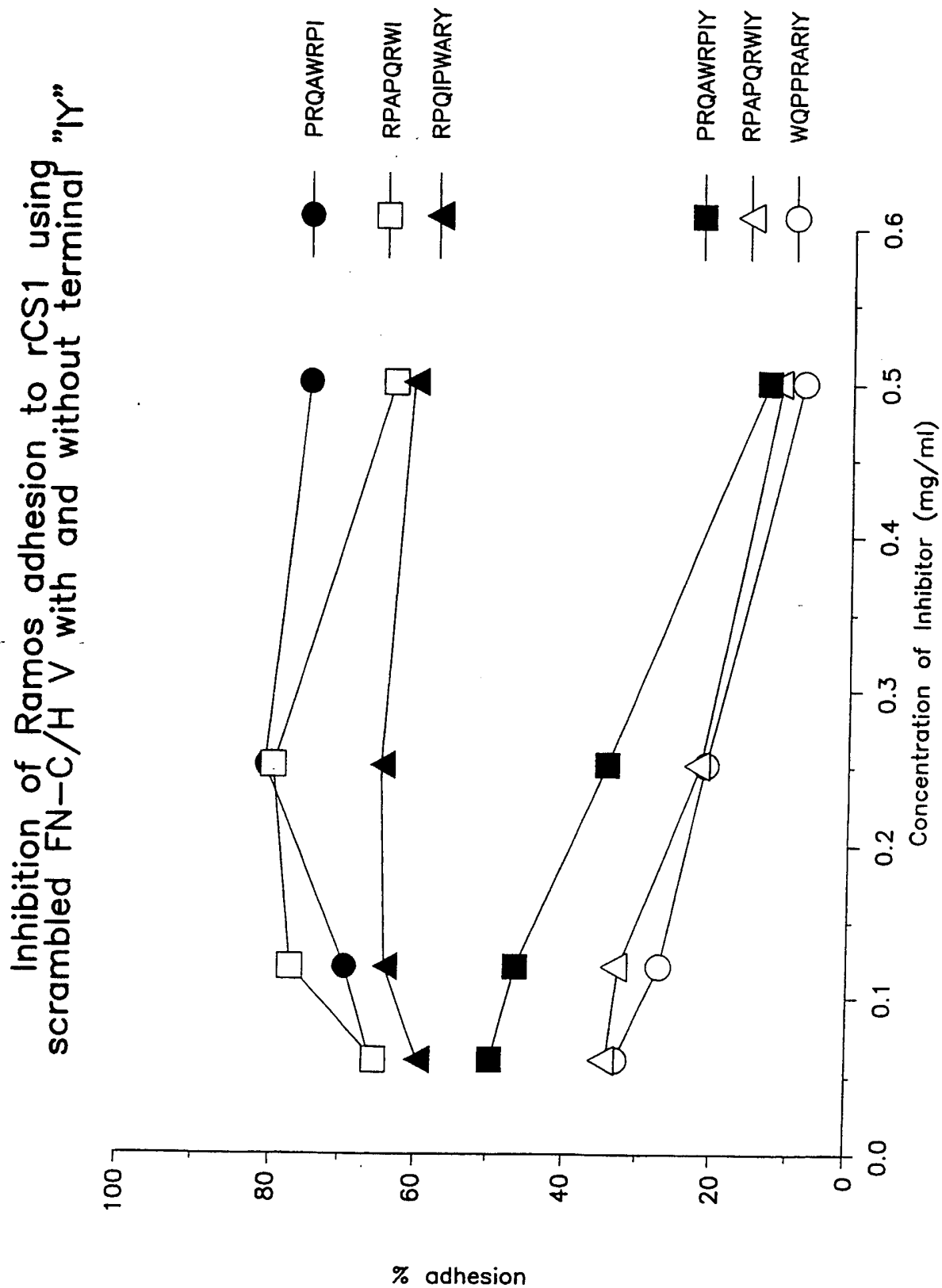


FIG. 5

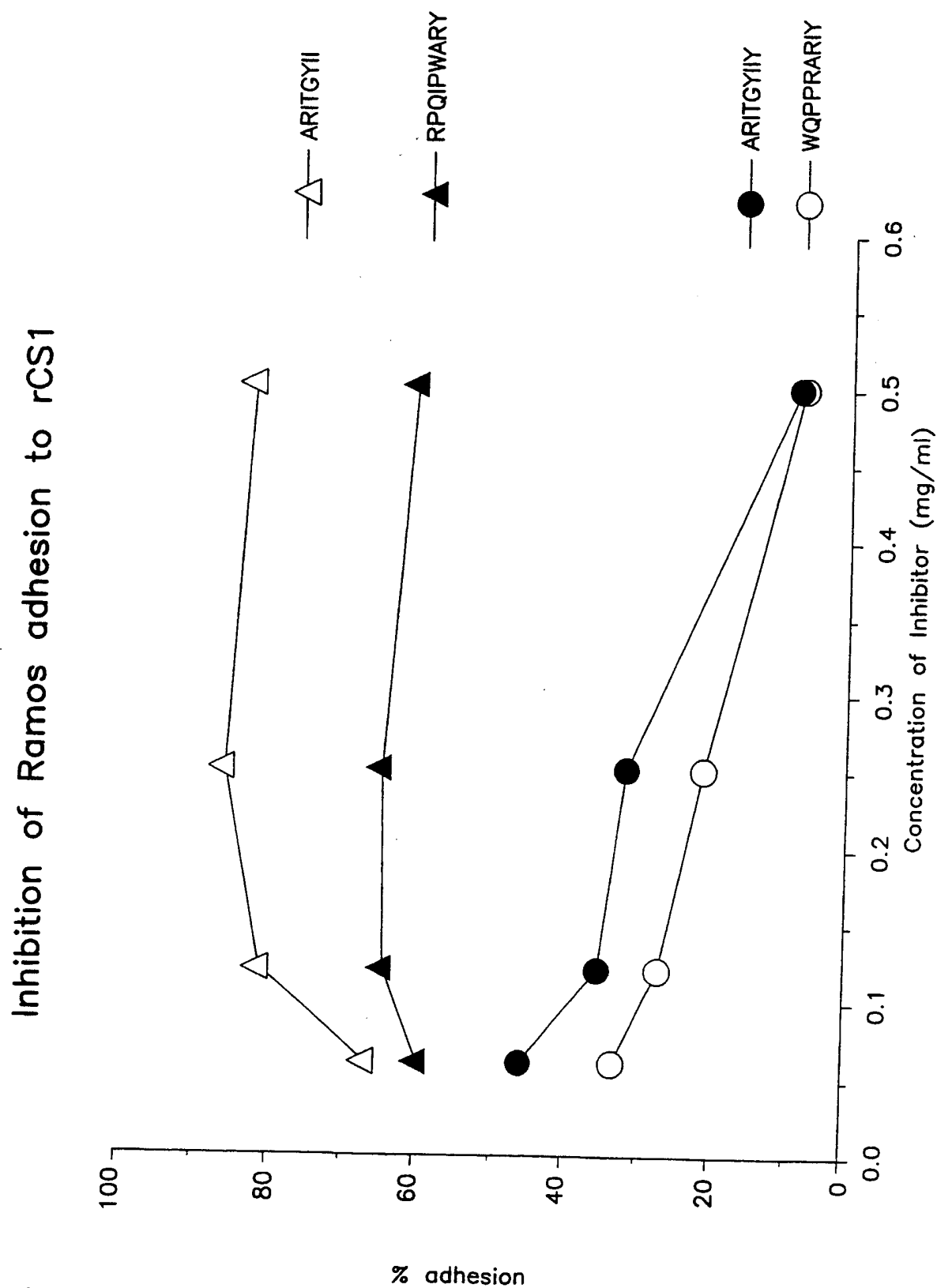


FIG. 6

FIG. 7
Inhibition of Ramos adhesion to rCS1 using deletions of peptide FN-C/H V

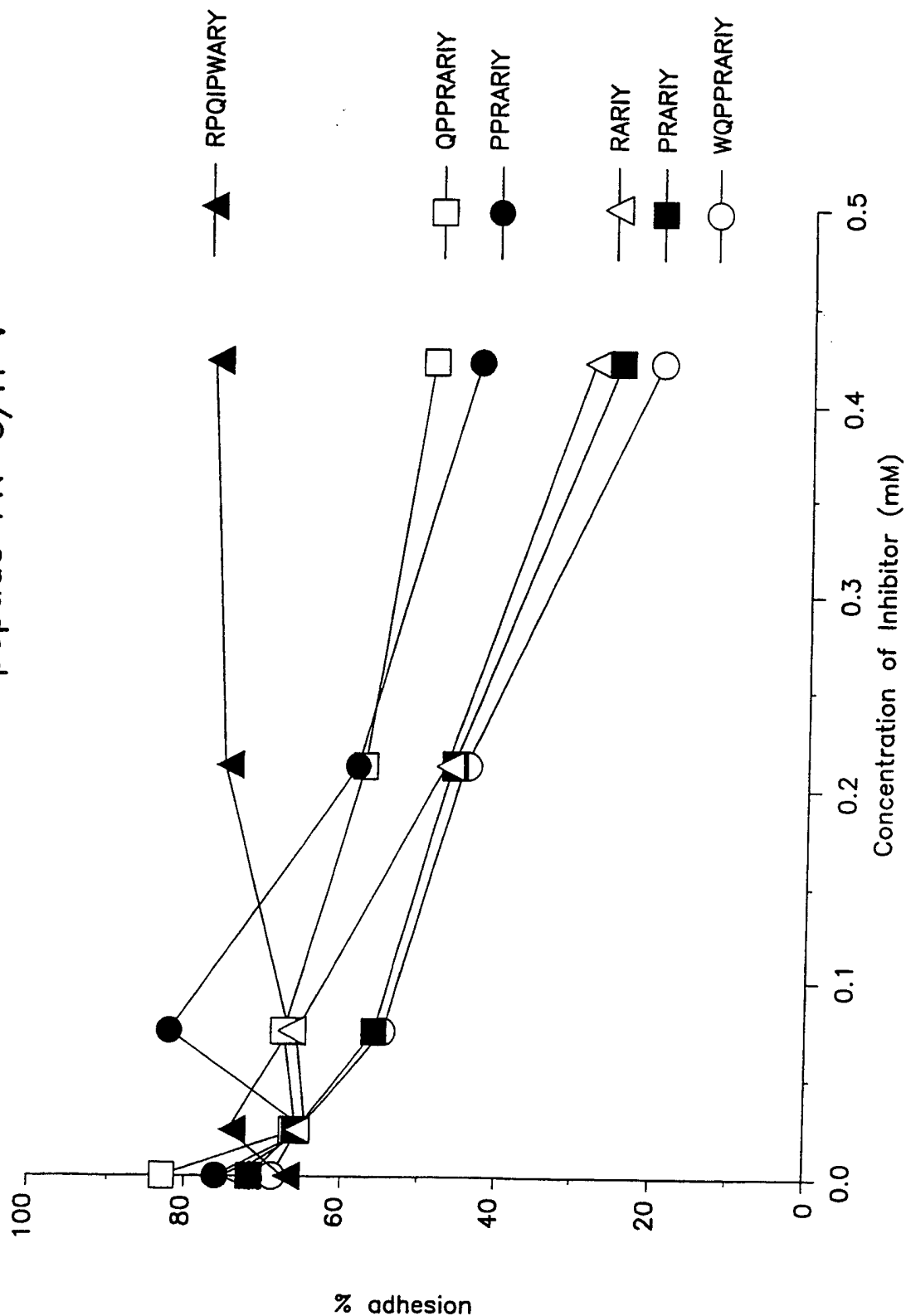


FIG. 8
Inhibition of Ramos adhesion to rCS1 using deletions of peptide FN-C/H V

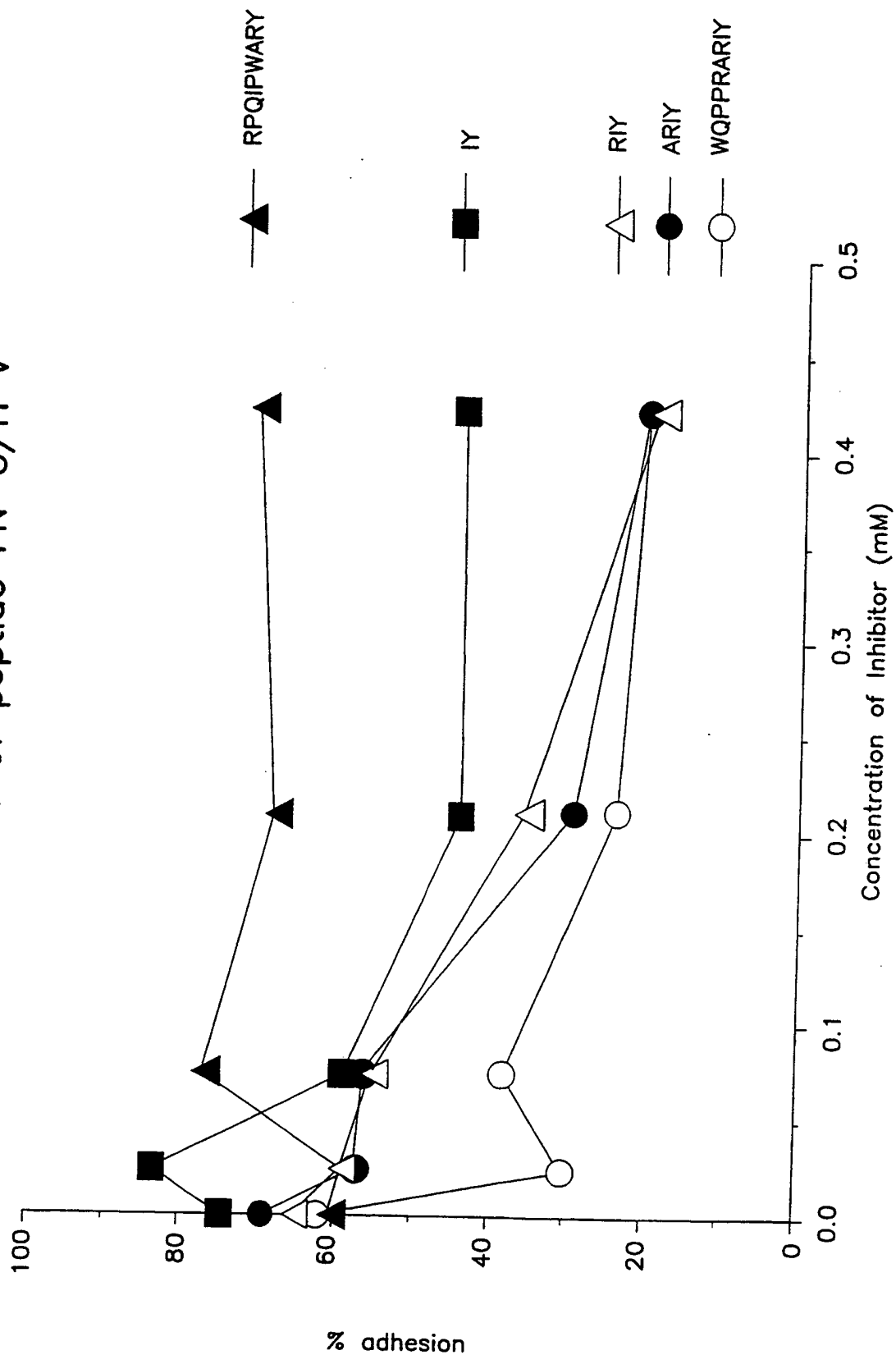
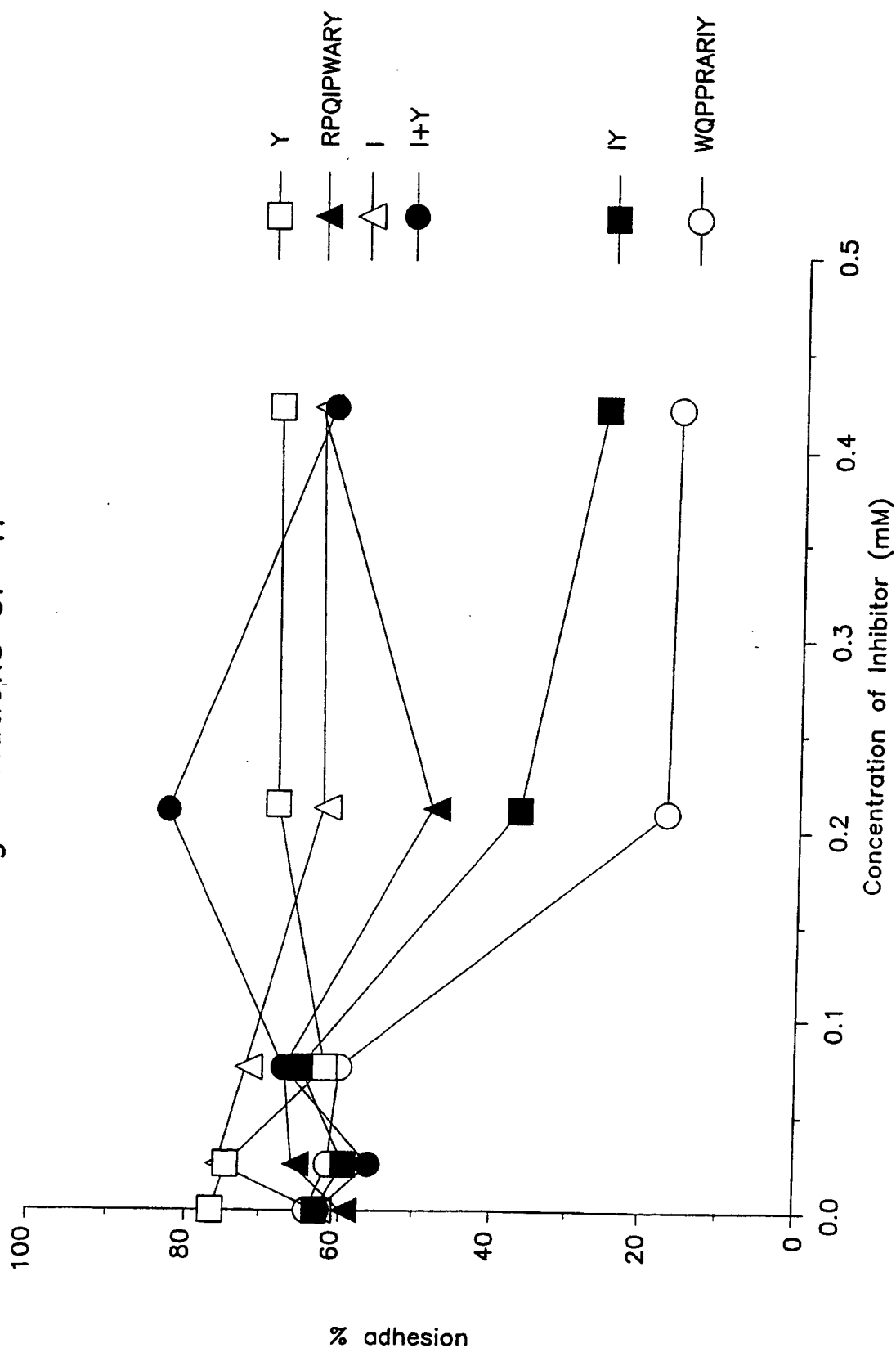


FIG. 9
Inhibition of Ramos adhesion to rCS1
using variations of "Y"



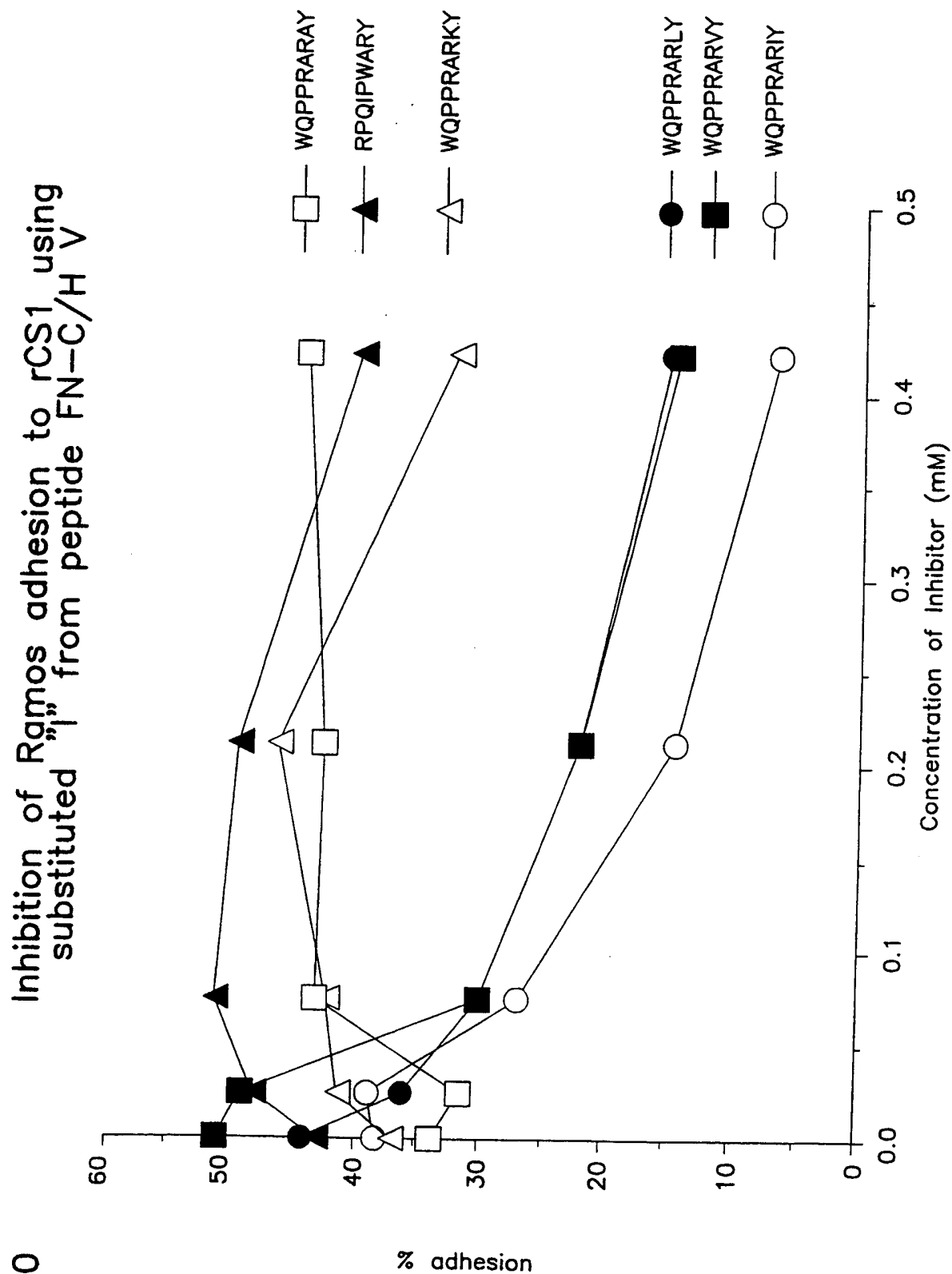


FIG. 10

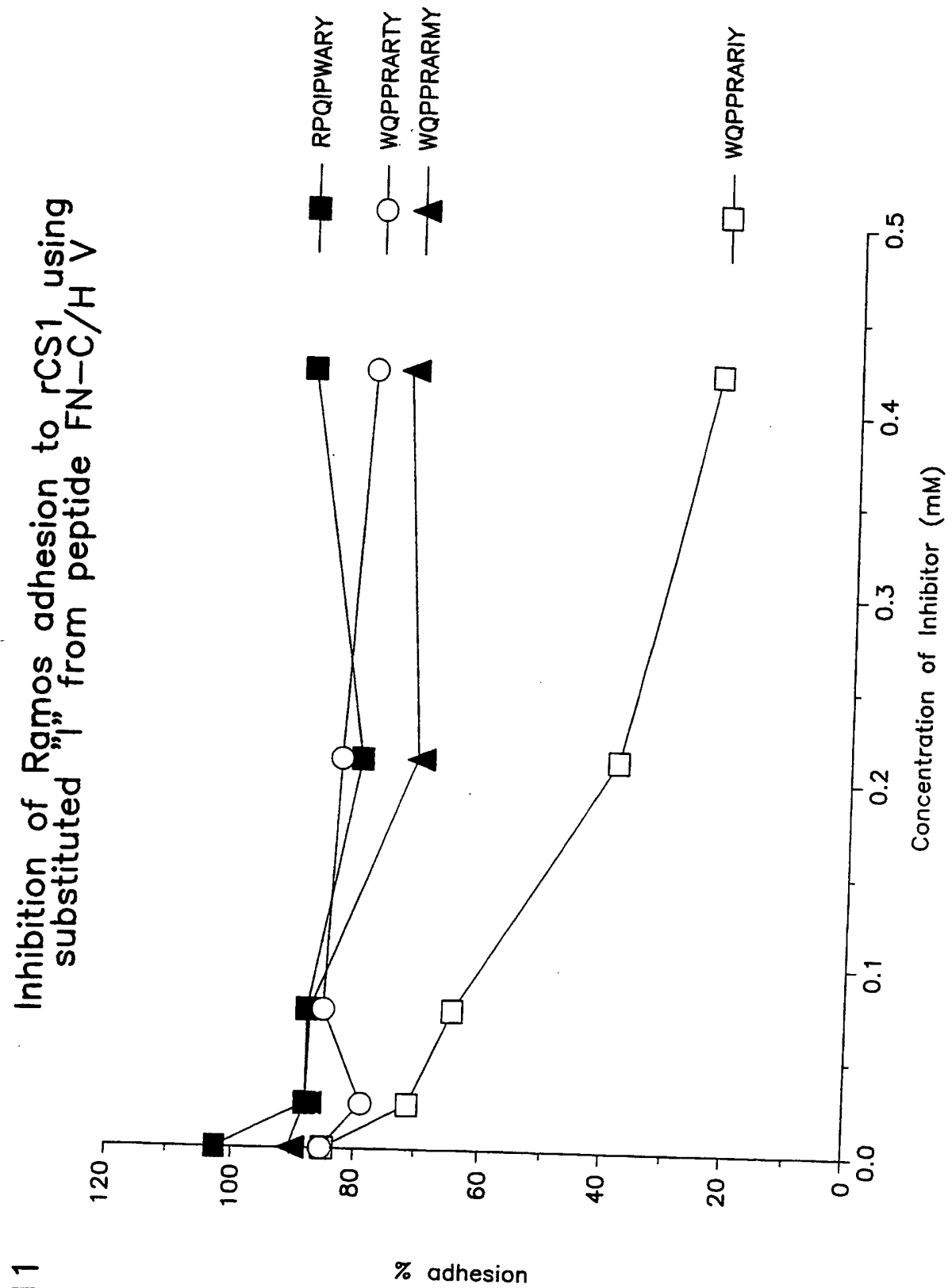


FIG. 11

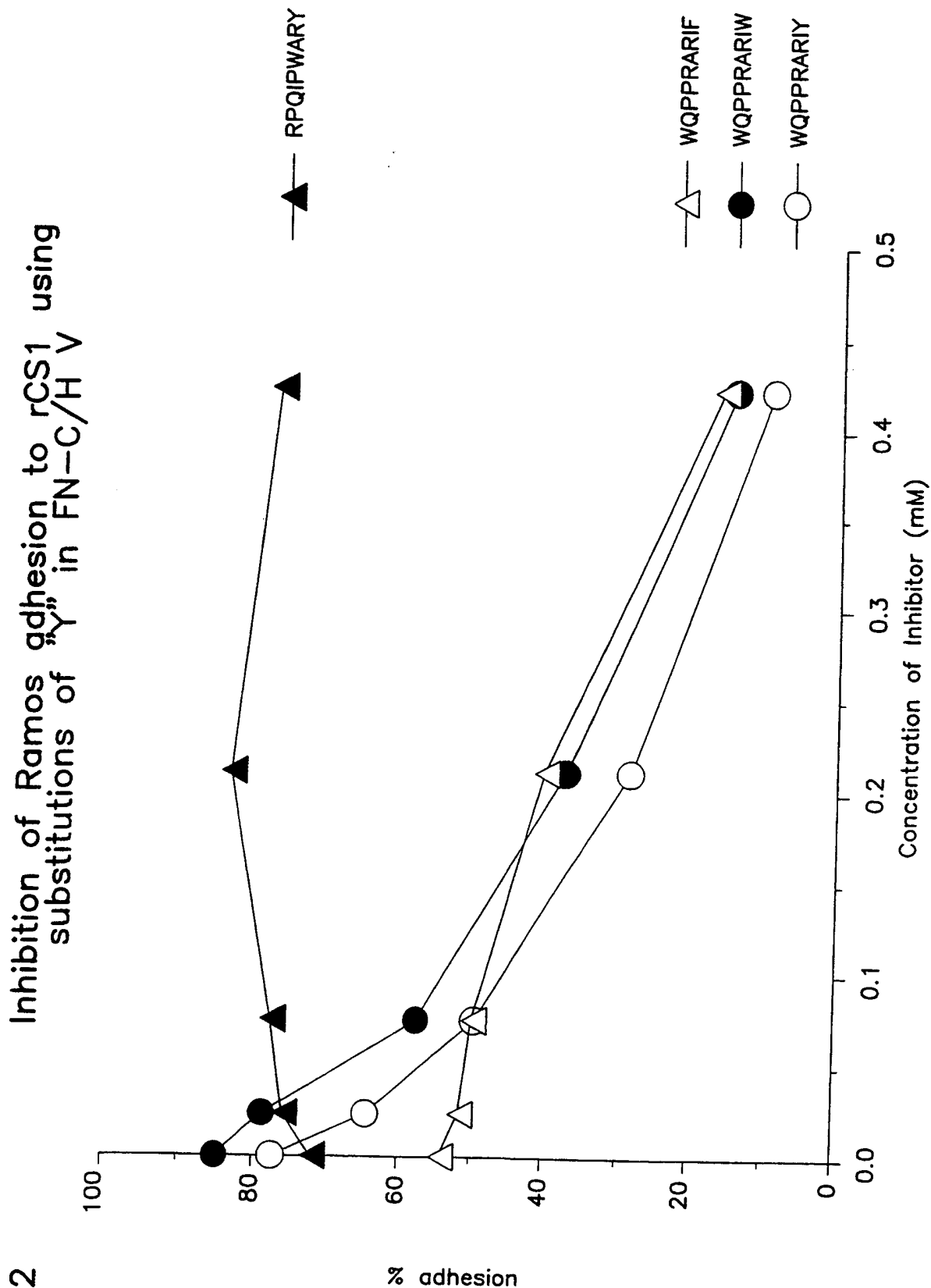


FIG. 12

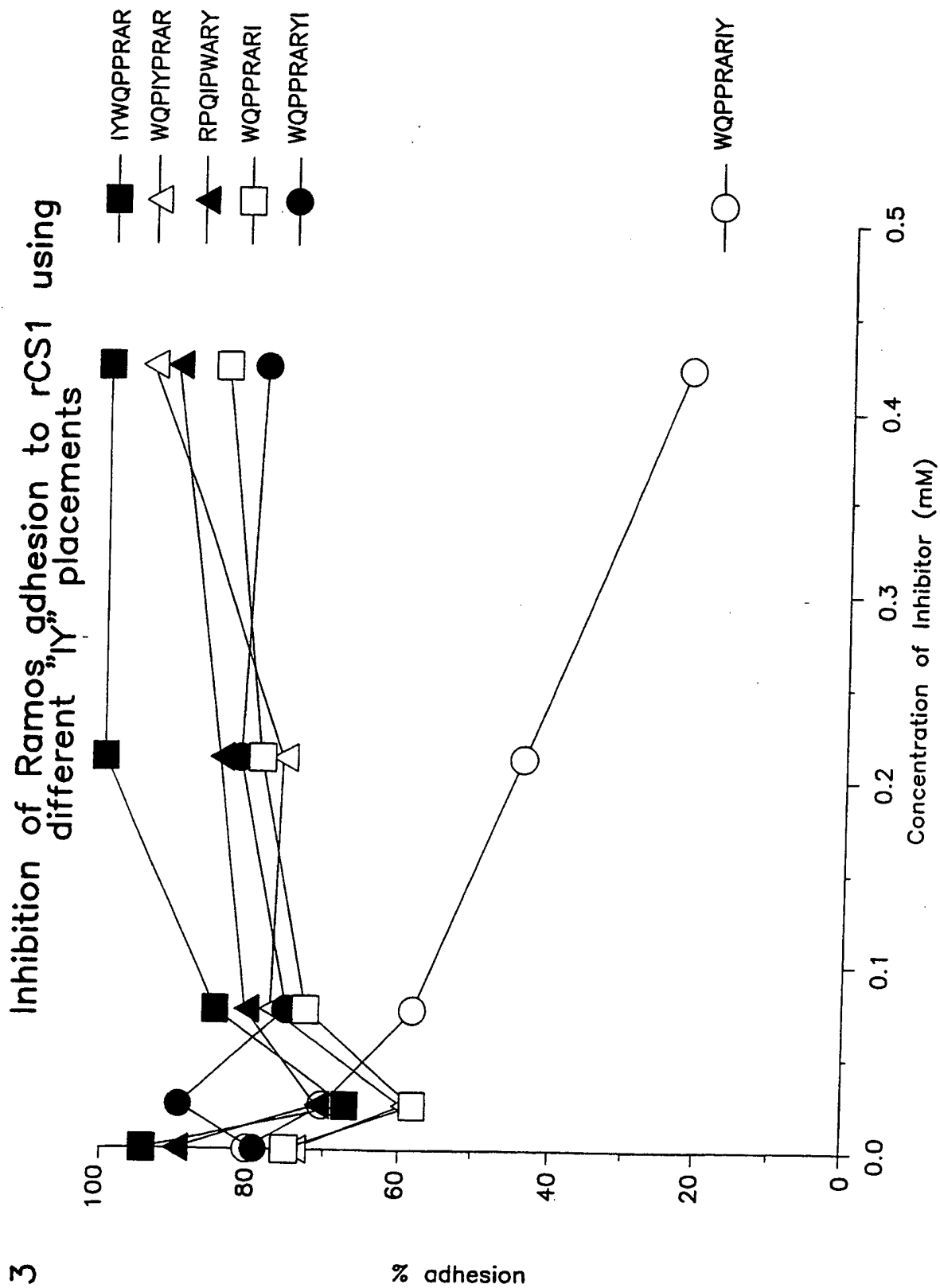


FIG. 13

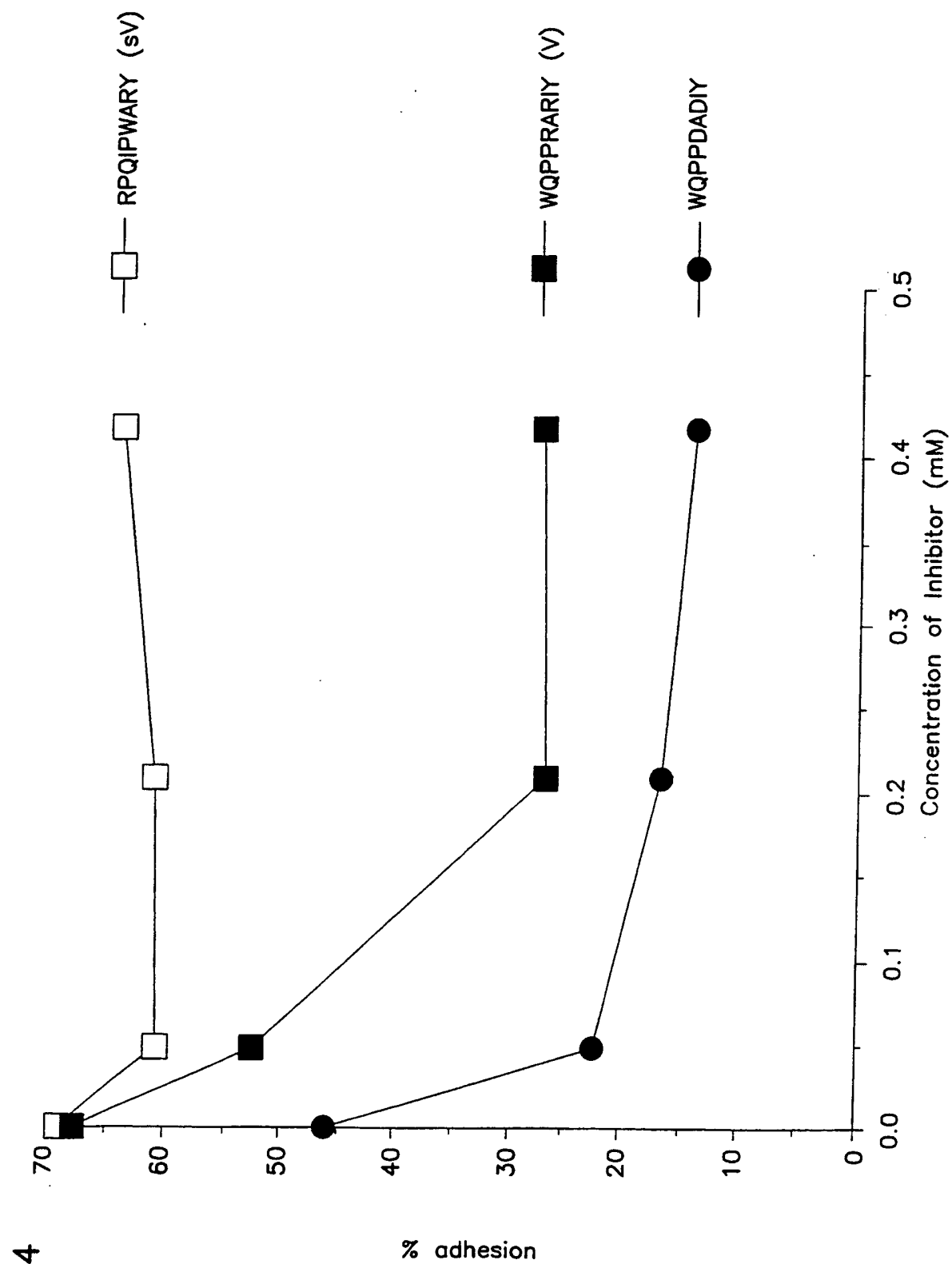
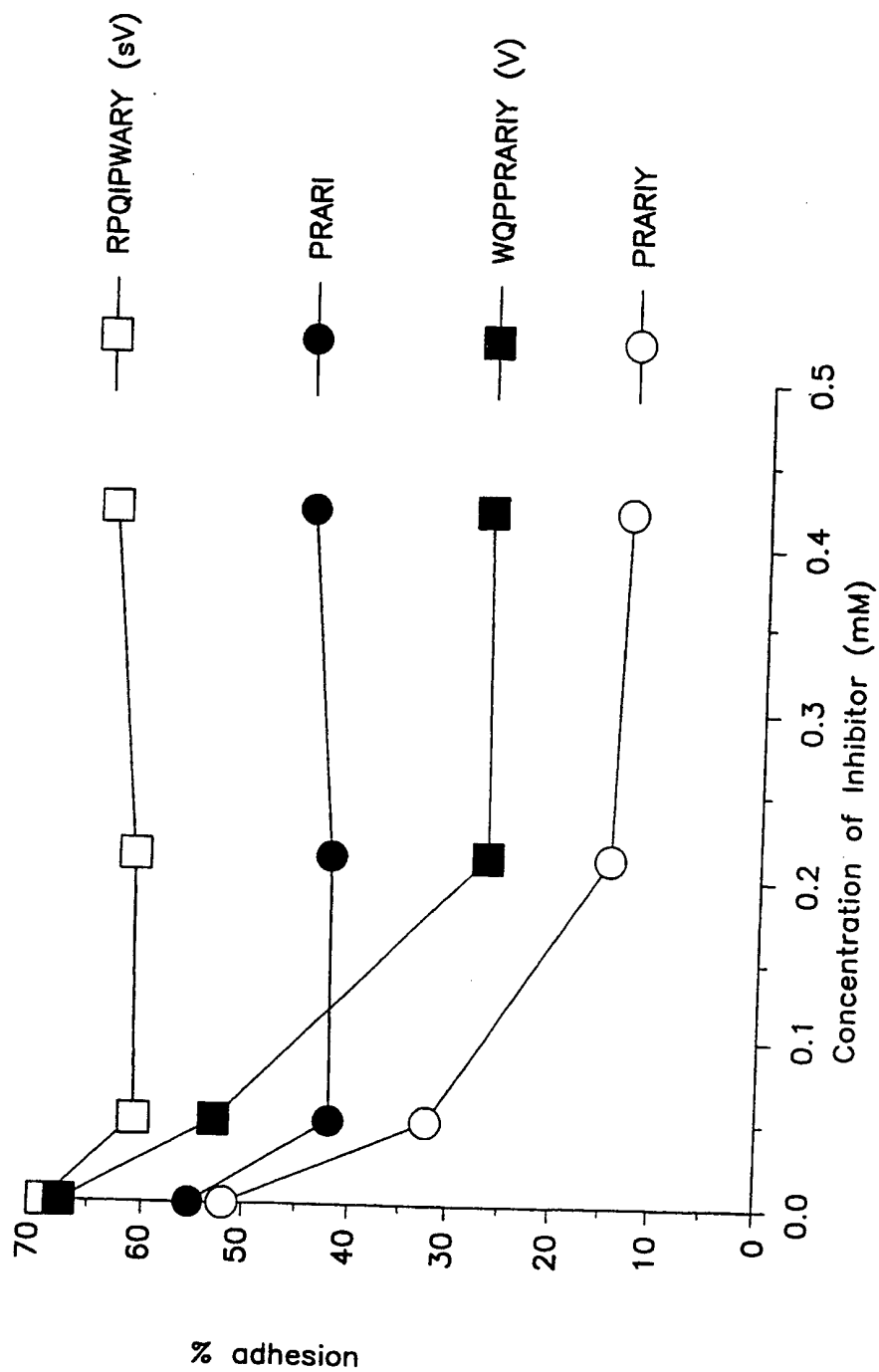


FIG. 15



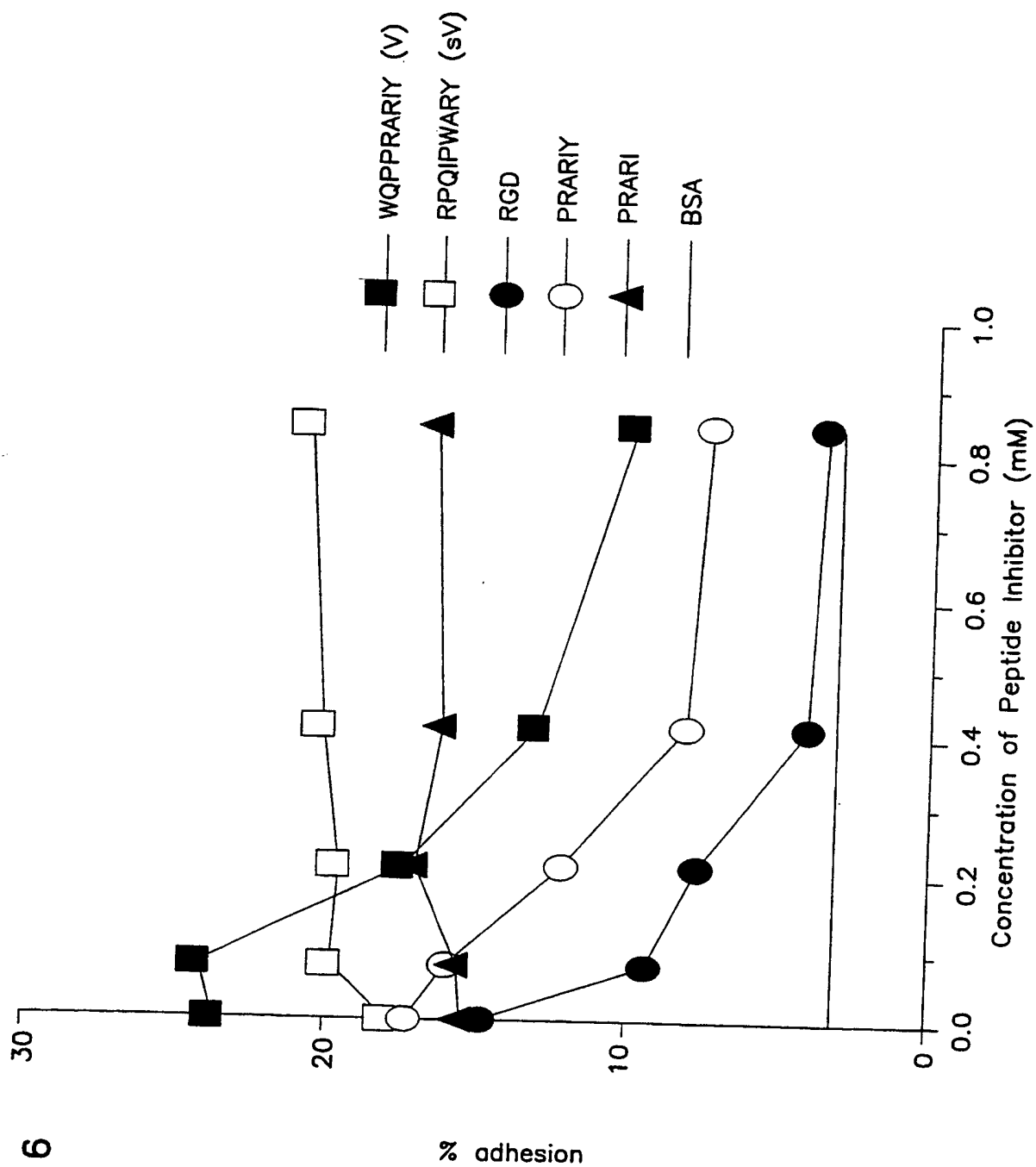


FIG. 16

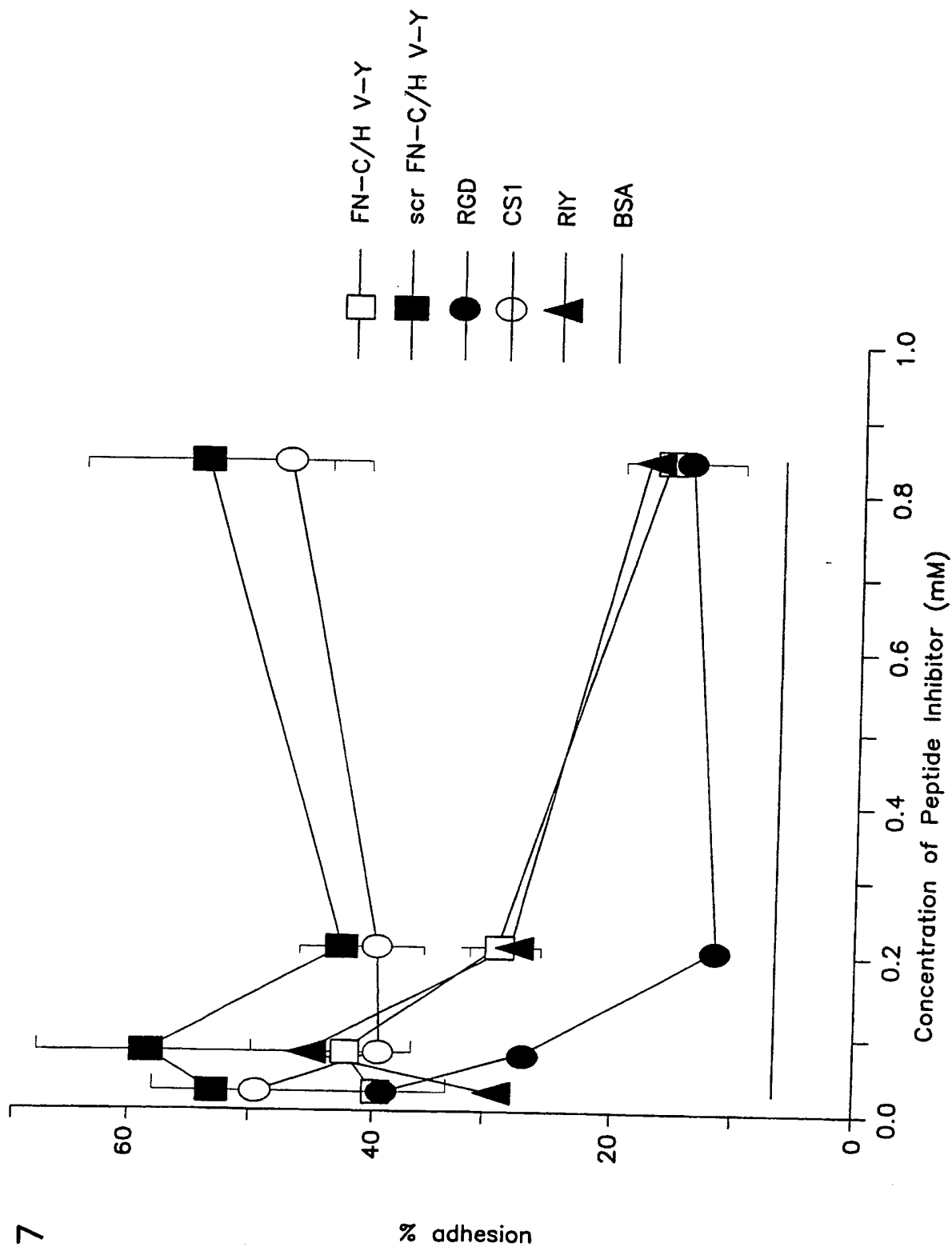
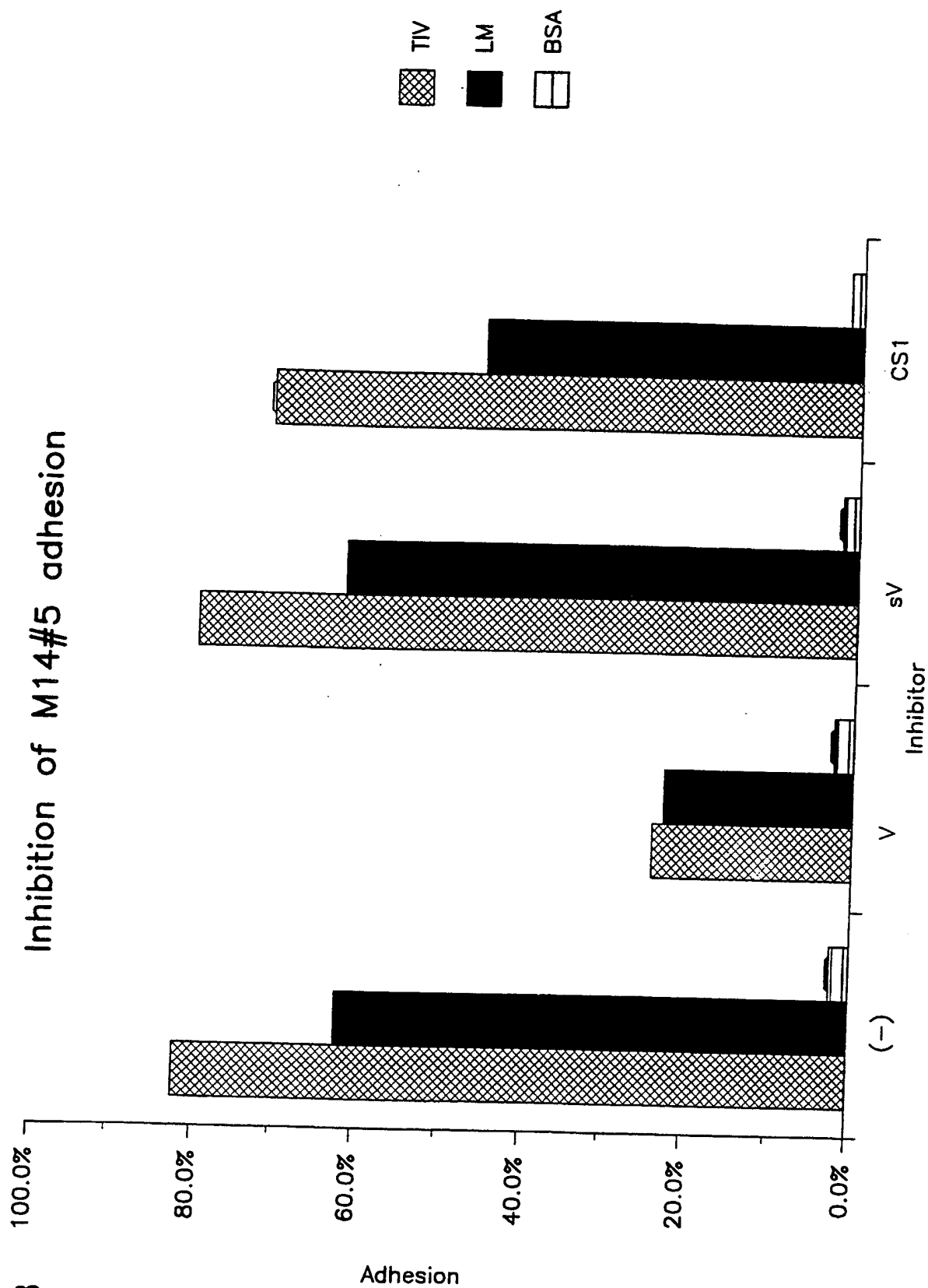


FIG. 17



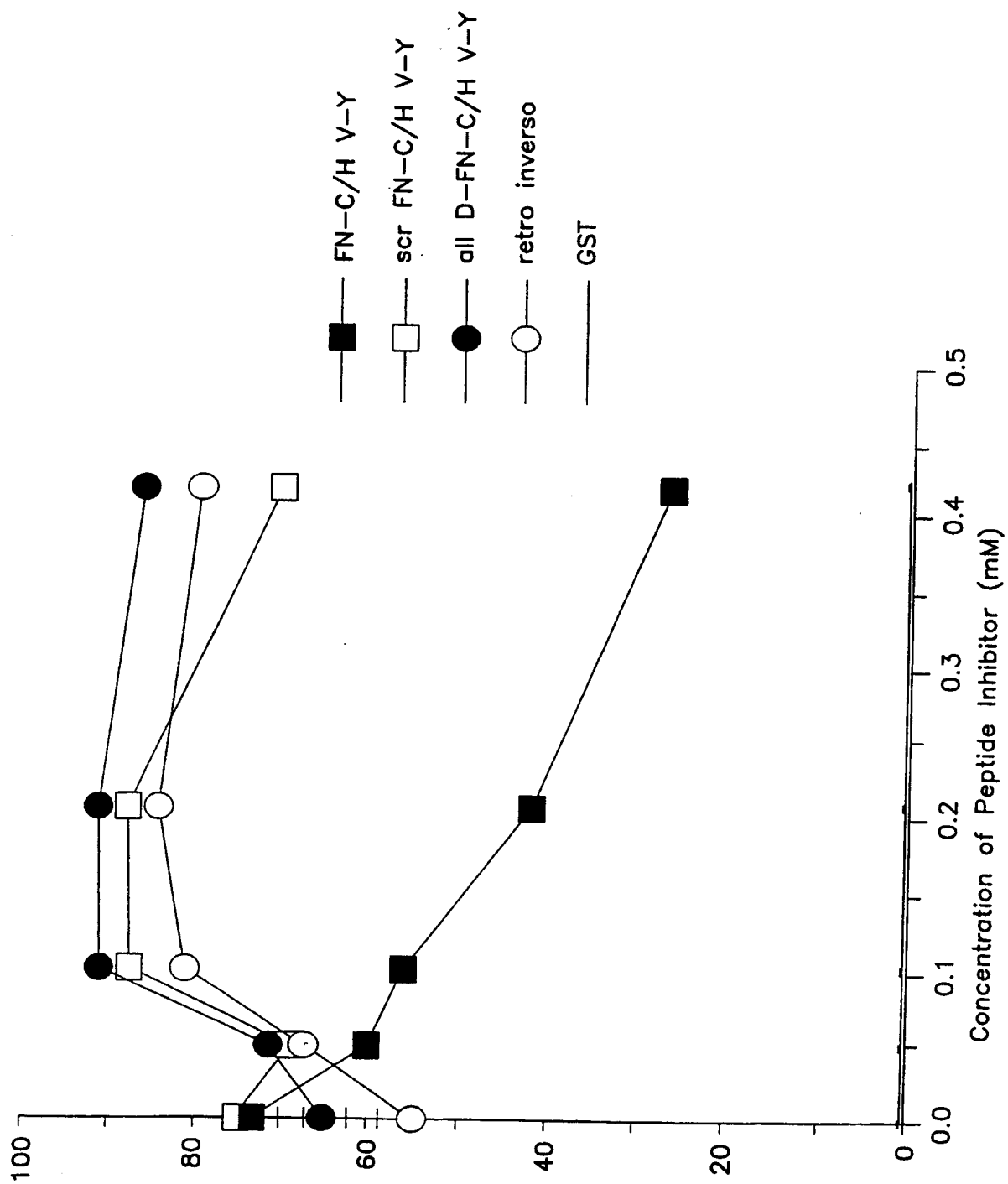


FIG. 19

INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No

PCT/US 99/01236

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 99/01236

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C07K7/06 C07K5/10 A61K38/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	J.L. LAUSER ET AL.: "INHIBITION OF MELANOMA CELL BINDING TO TYPE IV COLLAGEN BY ANALOGS OF CELL ADHESION REGULATOR" J. MED. CHEM., vol. 40, 1997, pages 3077-3084, XP002107028 see abstract	1-4, 7-12, 14, 23
X	WO 89 01942 A (UNIV MINNESOTA) 9 March 1989 see abstract; table III	12, 14
X	WO 94 17097 A (UNIV MINNESOTA ;US ARMY (US)) 4 August 1994 see page 1 see page 7, line 25 - line 26	12, 14
	-/--	



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents:

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Date of the actual completion of the international search

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Date of mailing of the international search report

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International Application No

PCT/US 99/01236

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>LASZ E C ET AL: "B3 INTEGRIN DERIVED PEPTIDE 217-230 INHIBITS FIBRINOGEN BINDING AND PLATELET AGGREGATION: SIGNIFICANCE OF RGD SEQUENCES AND FIBRINOGEN AA-CHAIN" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 190, no. 1, 15 January 1993, pages 118-124, XP000327808 see abstract see page 123, paragraph 1</p>	12,14
X	<p>EP 0 567 898 A (MANTHEY JUERGEN DR) 3 November 1993 see abstract</p>	12,14
X	<p>US 5 382 569 A (CODY WAYNE L ET AL) 17 January 1995 see column 61, line 10 - line 15; claims</p>	1-5
X	<p>EP 0 347 890 A (MORISHITA PHARMA ;AJINOMOTO KK (JP)) 27 December 1989 see page 15; example 12; table 15</p>	1,4,6